

# **STRUCTURE AND ORDERING IN AQUEOUS AMPHIPHILE SYSTEMS STUDIED BY NMR**

**Jan Ulmius**

**Lund 1978**





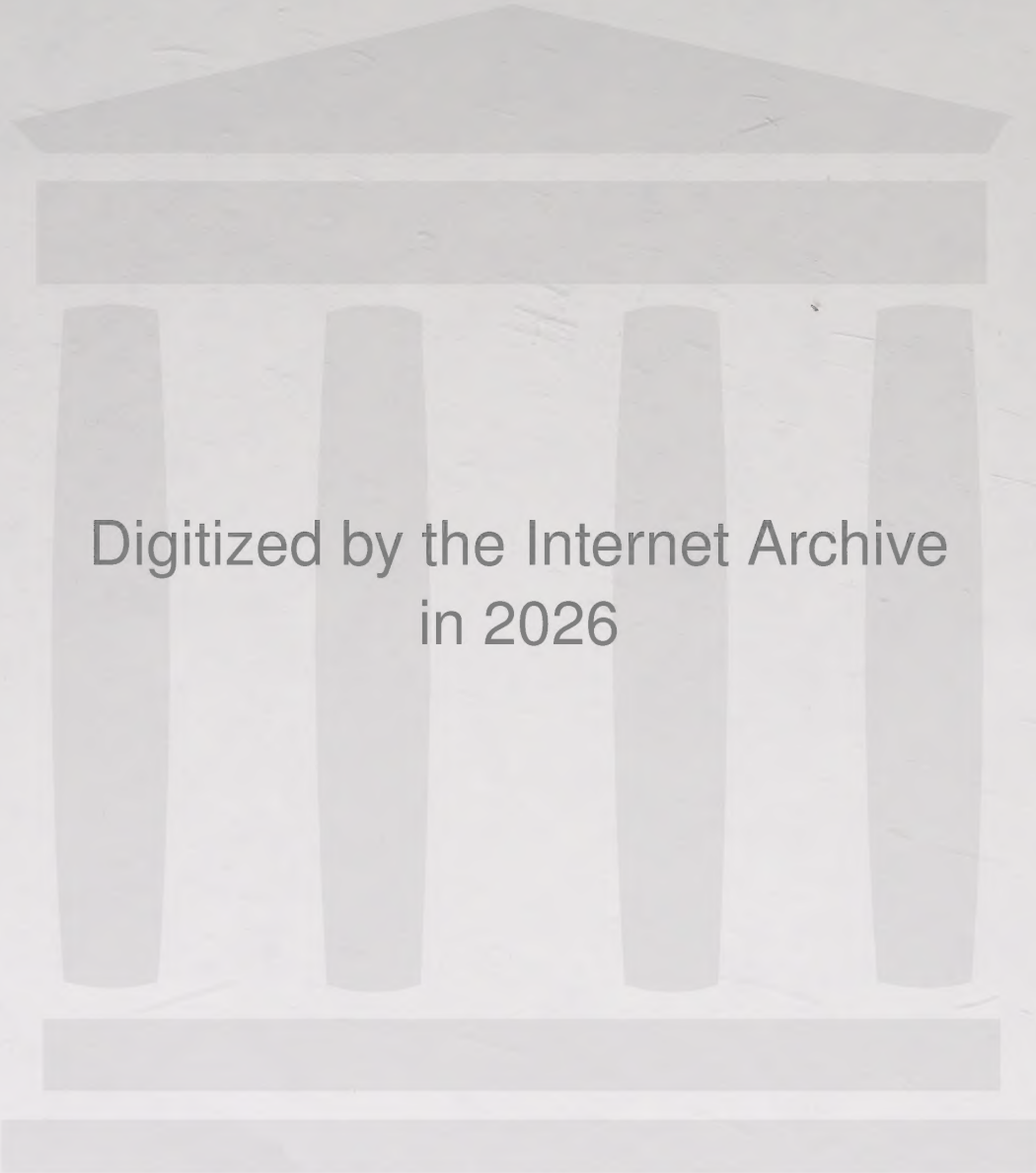


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STUDIED BY NMR

Jan Ulmius  
Civ.ing., Ld

AVHANDLING FÖR TEKNISK DOKTORSEXAMEN

Avhandlingen kommer att försvaras vid en offentlig  
disputation i sal D, Kemicentrum, Lund  
lördagen den 7 oktober 1978 kl. 10.15.



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STRUCTURE AND ORDERING IN  
AQUEOUS AMPHIPHILE SYSTEMS  
STUDIED BY NMR

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LUND 1978



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Dokumenttitel och undertitel

Structure and Ordering in Aqueous Amphiphile Systems Studied by NMR

Referat (sammandrag)

Lecithin lamellar liquid crystalline phases give superlorentzian 1-H NMR bandshapes. Simulation of these bandshapes is possible taking into account the mesophase symmetry and the lateral diffusion. Order parameters and the influence of cholesterol are discussed.

The slow rotational motion of micellar aggregates is shown to give rise to a 1-H NMR signal which is a superposition of lorentzian curves with different widths.

The bandshapes can be used to characterize the size of the aggregates.

2-H NMR studies of dipalmitoyl lecithin-water systems are used to construct a phase diagram. The very narrow 2-H splitting of water observed between the pretransition and the main transition is discussed.

2-NMR are also used to study water-binding, influence of ions and phase structure for Acholeplasma laidlawii lipids.

1-H and 2-H NMR and NMR diffusion measurements of the system lecithin-sodium cholate water give information about the structures for the different liquid crystalline phases.

Micellar aggregates in viscoelastic solutions of hexadecyltrimethylammonium salts are characterized by 1-H NMR and linear dichroism studies. Periodic colloidal structures are suggested responsible for the viscoelastic behaviour.

Referat skrivet av  
förf.

Förslag till ytterligare nyckelord

Klassifikationssystem och -klass(er)

Indextermer (ange källa)



Omfång

Övriga bibliografiska uppgifter

Språk  
English

Sekretessuppgifter

ISSN

ISBN

Dokumentet kan erhållas från  
Division of Physical Chemistry 2  
Chemical Center, P.O.Box 740  
S-220 07 Lund, Sweden

Mottagarens uppgifter

Pris

Blankett LU 11:25 1976-07

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Structure and Ordering in Aqueous Amphiphile Systems  
Studied by NMR

This thesis is based on the following publications, which are referred to by Roman numerals in the text.

- I. Proton NMR Bandshape Studies of Lamellar Liquid Crystals and Gel Phases Containing Lecithins and Cholesterol  
J. Ulmius, H. Wennerström, G. Lindblom and G. Arvidson, Biochim. Biophys. Acta 389, 197 (1975)
- II. Proton NMR Bandshapes for Large Aggregates; Micellar Solutions of Hexadecyltrimethylammonium Bromide  
J. Ulmius and H. Wennerström, J. Magn. Resonance 28, 309 (1977)
- III. Deuteron Nuclear Magnetic Resonance Studies of Phase Equilibria in a Lecithin-Water System  
J. Ulmius, H. Wennerström, G. Lindblom and G. Arvidson, Biochemistry 16, 5742 (1977)
- IV. Water Binding and Phase Structures for Different Acholeplasma laidlawii Membrane Lipids Studied by Deuteron Nuclear Magnetic Resonance and X-ray Diffraction  
Å. Wieslander, J. Ulmius, G. Lindblom and K. Fontell, Biochim. Biophys. Acta, in press
- V. The Liquid Crystalline Phases of the Lecithin-Sodium Cholate-Water-System. Molecular Organization Studied by NMR and Low Angle X-ray Diffraction Methods.  
J. Ulmius, G. Lindblom, H. Wennerström, K. Fontell, L.B.-Å. Johansson and G. Arvidson, to be published
- VI.  $^1\text{H}$ ,  $^{13}\text{C}$ ,  $^{35}\text{Cl}$  and  $^{81}\text{Br}$  NMR of Aqueous Hexadecyltrimethyl ammonium Salt Solutions: Solubilization, Viscoelasticity and Counterion Specificity,  
J. Ulmius, B. Lindman, G. Lindblom and T. Drakenberg, J. Colloid Interface Sci. 65, 88 (1978)



VII. Viscoelasticity in Surfactant Solutions. Characterization of the Micellar Aggregates and the Formation of Periodic Colloidal Structures.

J. Ulmius, H. Wennerström. L.B.-Å. Johansson, G. Lindblom and S. Gravsholt, to be published



## 1. INTRODUCTION

Applications of nuclear magnetic resonance spectroscopy to the study of chemical systems have grown enormously during the last two decades, extending from routine analytical purposes to imaging of the whole human body for diagnostic purposes.

In the present thesis nuclear magnetic resonance methods are applied to aqueous amphiphile systems. These systems occur in (and are a prerequisite for) living systems. Examples are cell membranes, lipoproteins, and bile lipids. Also the technical applications of these systems are innumerable, including detergency, pharmacy, catalysis, flotation and petroleum recovery. The information that can be obtained about these systems by nuclear magnetic resonance methods includes ordering and dynamics of the hydrocarbon chains of the amphiphile molecules, counterion binding and hydration of the amphiphile aggregates as well as diffusion (1-4).

### 1.1 Micelle formation

Substances possessing at the same time hydrophilic, i.e. water attracting, and hydrophobic, i.e. water repelling, parts are generally termed amphiphiles. The hydrophobic part is most commonly a hydrocarbon chain, while the hydrophilic part (head group) can vary considerably, with ionic, zwitterionic or dipolar groups. In Figure 1.1 some typical amphiphile molecules are displayed. The dual nature of amphiphilic molecules is responsible for the formation of different aggregates when the substance is dissolved in water.

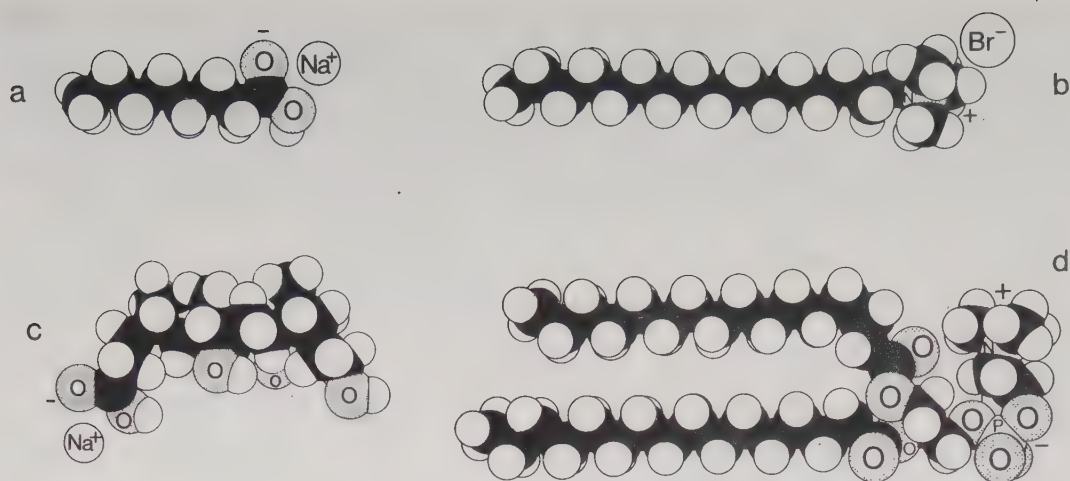


Figure 1.1. Space filling molecular models of a: sodium octanoate, b: cetyltrimethylammonium bromide (CTAB), c: sodium cholate, d: dipalmitoylphosphatidylcholine.

At low concentrations amphiphile molecules exist mostly as monomers and behave as simple electrolytes. At higher concentrations, numbers of molecules associate to form higher aggregates called micelles. This minimizes the energetically unfavourable exposure of the hydrocarbon chains to water. The micelles thus can be considered as consisting of a hydrocarbon core with the polar head groups facing outwards in the surrounding aqueous solution (Figure 1.2).

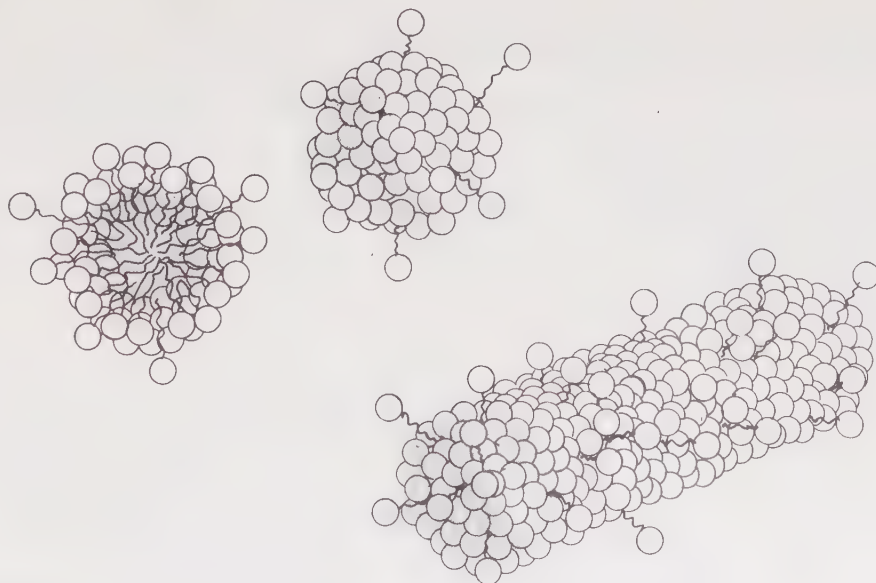


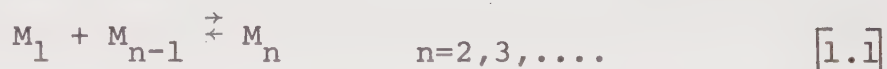
Figure 1.2. Schematic representation of spherical and rod-like micelles.



The interior of the micelle is almost like a liquid hydrocarbon droplet. The shape of the micelle is determined by the relative size of the alkyl chains and the polar head. A large polar group favours a spherical micelle. The electrostatic repulsion between charged amphiphile headgroups results in a large polar group area, and ionic amphiphiles generally form small spherical micelles. However, if the charged head groups are screened from each other, or in the case of nonionic amphiphiles, the polar head group area will be smaller and the micelle tends to be rod-shaped. (Figure 1.2). Rod-shaped micelles can grow to very large sizes, resulting in a broad size distribution (25).

A most useful property of micelles is their ability to solubilize, i.e. to dissolve hydrophobic material in their interiors, which has led to the use of micelle forming substances as detergents.

A general description of micelle formation is contained in the multiple equilibrium model. Here monomers associate in steps to form aggregates of various sizes according to the scheme:



This description is also supported by kinetic measurements where two greatly different time constants are observed, a rapid one corresponding to monomer exchange between micelles and bulk solution and a slow one, involving many steps, corresponding to the formation or dissolution of micelles. (4,5).

A simplified description of amphiphile aggregation is given by the phase separation model. For amphiphiles with hydrocarbon chains longer than about 6 carbons, a number of properties (i.e. electrical conductivity, surface tension and light scattering) show an abrupt change around a particular concentration, defined as the critical micelle concentration (c.m.c.). The monomer concentration increases with increasing

amphiphile concentration until the c.m.c. is reached, whereupon micelles start to form. Above the c.m.c. the monomer concentration is constant and further addition of the amphiphile only increases the concentration of micelles.

## 1.2 Liquid Crystalline Phases

If the concentration of amphiphile is increased to the solubility limit, in general the amphiphile will not precipitate from the solution, but instead a liquid crystalline phase or amphiphilic mesophase will form. Liquid crystals have properties intermediate between the ordered crystalline state and the liquid state. The liquid crystalline phases formed by amphiphiles are characterized by a lack of short range order, i.e. the hydrocarbon chains are in a liquid-like state, while there simultaneously exists a long range "crystalline" order in one, two or three dimensions.

In section 1.1 it was mentioned that rod-shaped micelles form under certain conditions. On increasing the amphiphile concentration these rods grow in length. This is the case for the system water-cetyltrimethylammonium bromide (CTAB)-hexanol shown in Figure 1.3. However, the micelles do not grow to in-

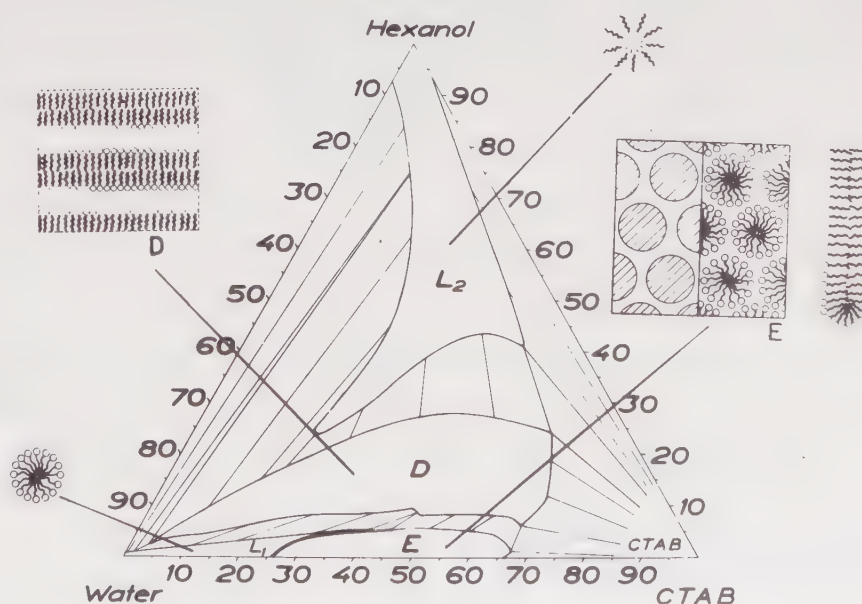


Figure 1.3. The ternary system water-cetyltrimethylammonium bromide-hexanol.



finity, because at a certain concentration the interaction between the micelles become important, and a phase transition occurs. The liquid crystalline phase that separates consists of long rods of amphiphile molecules arranged in a hexagonal lattice with water molecules surrounding the aggregates (Figure 1.4a).

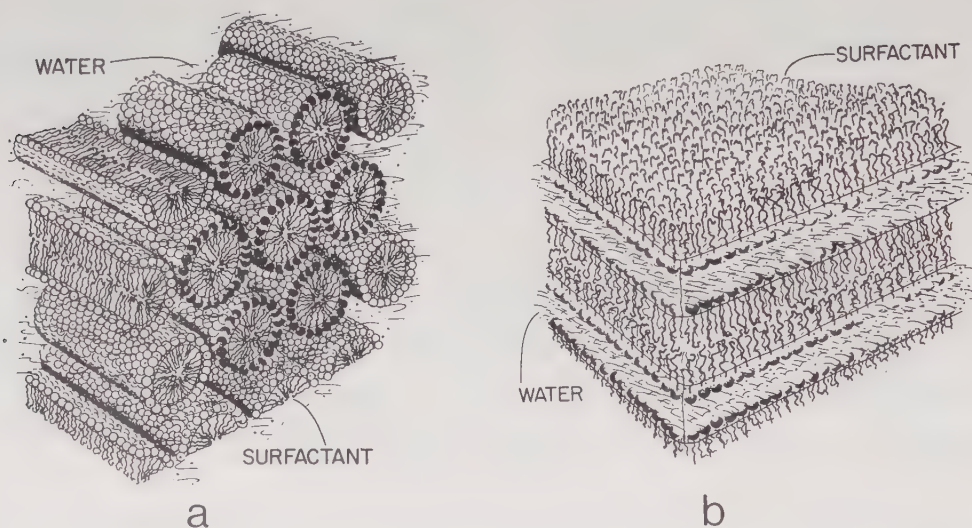


Figure 1.4. Schematic representation of the structures of a: the hexagonal mesophase and b: the lamellar mesophase.

As mentioned in section 1.1 the shape of micelles is determined by the relative size of the alkyl chains and the polar head. In the system water-CTAB-hexanol the geometric effect of hexanol is to increase alkyl chain volume, without appreciably changing the polar head group area. Hexanol has a long hydrocarbon chain but only a small polar group. This will favour a disc shaped micelle, but, as this grows in two dimensions, it is not stable, and a phase transition to a lamellar liquid crystalline phase will occur. It consists of bilayers of amphiphilic molecules, which are separated by layers of water as shown in Figure 1.4b.

### 1.3 Biological membranes

Every living cell is enclosed by a membrane that serves not only as a strong envelope inside which the cell can function,

but also as a discriminating gate, through which nutrients and other essential agents can enter and waste products can leave. Biological membranes are composed almost entirely of two classes of molecules: proteins and lipids (amphiphiles). The proteins provide the membrane with its distinctive functional properties. The lipids provide the structural properties of the membrane, and the most important class of lipids is the glycerophospholipids. A glycerophospholipid molecule has a glycerol backbone with two hydrocarbon chains and a polar head group attached (Figure 1.1). This arrangement favours a lamellar structure, and the now accepted model of the biological membrane is a lipid bilayer matrix in which the proteins are embedded in some way (Figure 1.5).

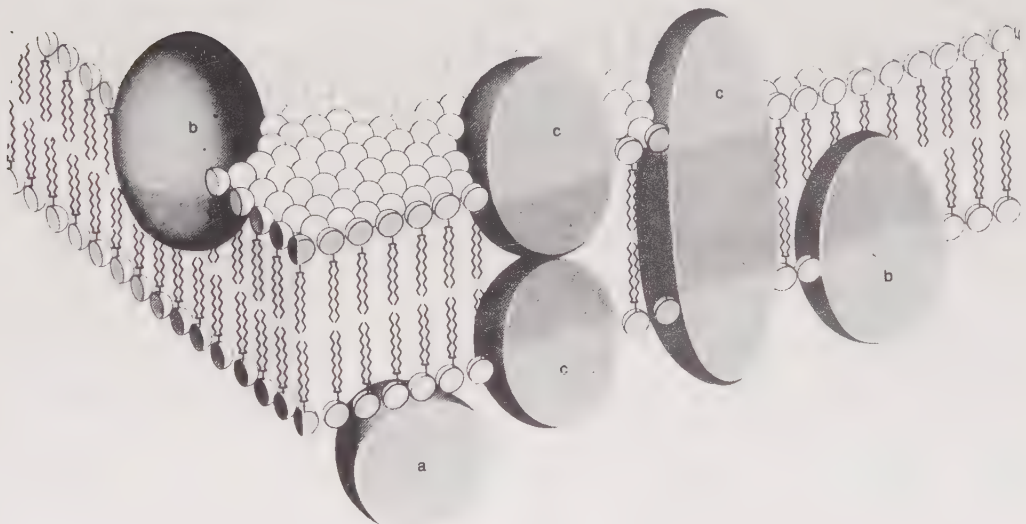


Figure 1.5. A schematic representation of a biological membrane.

#### 1.4 Nuclear Magnetic Resonance Methods

In nuclear magnetic resonance (NMR) spectroscopy, transitions between the nuclear spin energy levels in a static magnetic field  $B_0$  are studied. The spin energy level arrangement is determined by a time independent static nuclear spin hamiltonian, and the parameters that enter this hamiltonian



are chemical shifts, spin-spin couplings, magnetic dipole couplings and quadrupole couplings.

In solids with very slow molecular motion, the static hamiltonian depends on the orientation of the molecules with respect to the magnetic field  $B_0$ . For a single crystal the NMR spectrum changes when the crystal is rotated in the magnetic field. For a polycrystalline sample, where all molecular orientations are present, the spectrum consists of superimposed spectra from all orientations, and a so called powder spectrum results.

When molecular motion becomes faster, the orientation of the molecules changes with time, and the magnitude of the static interactions is given by the time average of the spin hamiltonian. In liquids with rapid molecular motion the time average of dipole couplings and quadrupole couplings is zero and no dipole- or quadrupole splittings are observed. However, the chemical shifts and the spin-spin coupling have a non-zero time average and a NMR spectrum that is dependent on the chemical structure can be observed. This dependence is the basis of the use of NMR as an analytical tool.

In liquid crystals with motional freedom only in certain directions, the time average of dipole and quadrupole interactions can be non-zero, and couplings can be observed in the NMR spectrum. They are, however, smaller than in the case of a rigid lattice. The couplings can give valuable information about molecular structure and order.

A time dependent hamiltonian with a zero time average can still be very important, because it can cause transitions between the energy levels, or with another name, relaxation. The time constant  $\tau$  characterizes this process, the relaxation time, can give information about molecular dynamics in the system. The relaxation times most frequently measured are the spin lattice relaxation time  $T_1$  and the spin-spin relaxation time  $T_2$ .  $T_1$  is the time constant that characterizes

the approach to thermal equilibrium through energy exchange with the thermal motions of the molecule and the surrounding molecules.  $T_2$  is the time constant for the establishment of the thermal equilibrium within the spin system. One can say that  $T_2$  is a measure of the uncertainty of finding the spin in a certain state, and is a measure of the bandwidth of the NMR signal. For molecular motions that are rapid compared to the nuclear resonance frequency (or Larmor frequency)  $T_1$  is equal to  $T_2$ .



## 2. PROTON NMR BANDSHAPES

### 2.1 Liquid Crystalline Systems

Proton NMR spectra of lamellar and hexagonal lyotropic liquid crystals show a characteristic bandshape, originally observed by Lawson and Flautt (6). This bandshape, called super-lorentzian, has a sharp central peak and broad intense wings. Wennerström (7) has presented an explanation for the origin of the observed bandshape starting from the symmetry of the liquid crystalline phase and the assumption that the lateral diffusion is fast enough to average the intermolecular static dipolar interactions to zero.

The dipolar part of the hamiltonian can be written

$$H_D = \sum_{i>j,q} (-1)^q F_{-q}^{(2)}(ij) A_q^{(2)}(ij) \quad [2.1]$$

where  $A_q^{(2)}(ij)$  is a spin tensor operator which it is convenient to express in a laboratory fixed coordinate system (L) which is chosen to make  $B_0 = B_z$  the only non-zero component of the magnetic field. The  $F_{-q}^{(2)}(ij)$  is a tensor whose components depend on the distance between and the orientation of the two nuclear magnetic dipoles  $i$  and  $j$ . It is thus a molecular quantity and best described in a molecular-fixed coordinate system (M).

The transformation between the different coordinate systems is easily performed because the different tensor components have simple transformation properties on rotation of the coordinate system. It is advantageous to perform the transformation in two steps (8). A director coordinate system D is defined which for a lamellar phase has the z-axis normal to the lamellae.

The transformation for  $A_q^{(2)}$  can now be written

$$A_q^{(2)M} = \sum_{q'} A_{q'}^{(2)D} D_{q'q}^{(2)}(\Omega_{DM}) \quad [2.2]$$

$$A_{q'}^{(2)D} = \sum_{q''} A_{q''}^{(2)L} D_{q''q'}^{(2)}(\Omega_{LD}) \quad [2.3]$$

where  $D^{(2)}$  are Wigner rotation matrix elements and  $\Omega$  are the eulerian angles that specify the rotations (9).

Combining eqs. [2.1] through [2.3] gives

$$H_D = \sum_{i>j, q, q'} (-1)^q F_{-q}^{(2)M} A_{q''}^{(2)L} (ij) D_{q''q'}^{(2)}(\Omega_{LD}) D_{q'q}^{(2)}(\Omega_{DM}) \quad [2.4]$$

The z-axis of the M-system is now chosen to lie along the i-j vector, making  $F_0^{(2)}(ij)$  the only non-zero component.

In a strong magnetic field only the secular part of eq.

[2.4] affects the spectrum which means that  $q''=0$ . The  $\Omega_{DM}$  changes with time due to molecular motion so that only the mean value of eq. [2.4] contributes to the static spin hamiltonian.

Now eq. [2.4] can be written

$$H_D = \sum_{i>j, q'} \overline{F_0^{(2)M} D_{q'0}^{(2)}(\Omega_{DM})} D_{0q'}^{(2)}(\Omega_{LD}) A_0^{(2)L} \quad [2.5]$$

If the lateral diffusion in the liquid crystalline state is fast, leading to zero average for intermolecular dipolar couplings it will also give the lamellae an effective cylindrical symmetry with the result that (8)

$$\overline{D_{q'0}^{(2)}(\Omega_{DM})} = \overline{\delta_{q'0} D_{q'0}^{(2)}(\Omega_{DM})} = 1/2 (3 \cos^2 \beta_{DM} - 1)$$

and also

$$D_{00}^2(\Omega_{LD}) = 1/2 (3 \cos^2 \beta_{LD} - 1)$$

and finally

$$H_D = 1/4 (3 \cos^2 \beta_{LD} - 1) \sum_{i>j} A_0^{(2)L} \overline{F_0^{(2)M} (3 \cos^2 \beta_{DM} - 1)} \quad [2.6]$$



The sum  $i > j$  in eq. [2.6] is now only over intramolecular pairs. If the chemical shift differences are small compared to the dipolar couplings, the shape of the absorption curve is independent of  $\beta_{LD}$ . The effect of a variation of  $\beta_{LD}$  is only a change in frequency scale since the couplings between all protons are multiplied by the same factor  $(3 \cos^2 \beta_{LD} - 1)$ .

Let us call the bandshape for a given  $\beta_{LD}$   $L(\nu - \nu_0, \cos \beta_{LD})$ , which is a symmetric function centered at the resonance frequency  $\nu_0$ .

In a liquid crystalline phase, no particular directions are favoured, and all angles between the director and the field,  $\beta_{LD}$ , are equally probable. The observed bandshape is then obtained by integrating over all the equally probable values of  $\cos \beta_{LD}$

$$L(\nu - \nu_0) = \int_{-1}^{+1} L(\nu - \nu_0, \cos \beta_{LD}) d \cos \beta_{LD} \quad [2.7]$$

If  $L(\nu - \nu_0, \cos \beta_{LD})$  is sizeable around  $\nu_0$ ,  $L(\nu - \nu_0)$  will have a shape with a sharp peak but still much intensity far out in the wings. If one has isolated proton pairs this condition is not satisfied and a normal powder spectrum will be observed (10). However, for a long alkyl chain,  $L(\nu - \nu_0, \cos \beta_{LD})$  will not have much fine structure since each proton has five near neighbours and the whole proton system becomes strongly coupled. By simply assuming a gaussian shape for  $L(\nu - \nu_0, \cos \beta_{LD})$  it is possible to reproduce spectra for lecithin-water systems, as is shown in paper I.

For lecithin systems, the isotropic shifts between the different types of protons must be taken into account, which in paper I is done by assuming that the signals from the different types of protons are independent and simply superimpose  $L(\nu - \nu_0, \cos \beta_{LD})$  of different widths. This should be a good approximation for the N-methyl protons, but not as good for the  $\omega$ -methyl protons.

The immediate practical consequence is that measurements of the width at half height ( $\Delta \nu_{1/2}$ ) in the spectrum are of little use to characterize the signal. To describe the observed signal a complete simulation must be performed. Such simulations give the width of the gaussian curve assumed for  $L(\nu, \cos\beta_{LD})$ . This width should be proportional to the interaction between two geminal methylene protons and thus give information about the order parameter. The order parameter can be used as a measure of the hydrocarbon chain ordering or the configuration of the fatty acyl chain.

However, it seems that when approximating the complex dipolar splitting pattern with a gaussian curve, the average dipolar splitting will be overestimated, leading to a too large average order parameter. The order parameter given in paper I should be compared with the value  $|S_{HH}| = 0.2$ , deduced from the deuterium order parameter  $S_{CD} = -0.25$  (11) for the upper part of the hydrocarbon chain in lecithin.

The basic reason for the difference between proton NMR spectra of the gel and the liquid crystalline phases can be found in the difference between the hamiltonians [2.5] and [2.6]. In the gel phase there is slow lateral diffusion and neither an effective cylindrical symmetry nor a zero average of intermolecular dipolar couplings is achieved.

In order to observe a spectrum with super-lorentzian shape the lateral diffusion coefficient must be greater than about  $10^{-13} \text{ m}^2/\text{s}$ , deduced from an assumed static intermolecular dipole-dipole coupling of 3 kHz. Lateral diffusion coefficients measured in the liquid crystalline state by different methods (12-14) are all centered around the value  $2 \cdot 10^{-12} \text{ m}^2/\text{s}$ . In the gel phase the lateral diffusion coefficient estimated by fluorescence techniques is less than  $10^{-14} \text{ m}^2/\text{s}$  (14).

Can the proton NMR bandshape of lecithin tell us something about the interactions between the lipid molecules and



other molecules, e.i. cholesterol and proteins, that are part of biological membranes? The role played by cholesterol in membranes has been under active investigation for many years, but is still far from clear. It is generally agreed that the addition of cholesterol to lipids in the gel phase 'fluidizes' the lipid fatty acid chains, whereas addition of cholesterol to lipids in the liquid crystalline phase has a 'condensing' effect (15).

In paper I it is shown that, after addition of cholesterol to lecithin, the proton NMR spectra below the transition temperature for the pure lecithin are super-lorentzian, and thus the lateral diffusion is higher than  $10^{-13} \text{ m}^2/\text{s}$  in the lecithin-cholesterol system. The same result is obtained with other methods (14). Above the phase transition for the pure lecithin there is no noticeable effect of cholesterol on the proton NMR spectra. It has, however, been shown that the order of the lipid fatty acid chains increases on addition of cholesterol (16, 17).

The question of phase heterogeneity and specific lecithin-cholesterol complexes is also complex and far from solved (17, 18).

The effect of membrane proteins on the bulk proton NMR signal of the lipids is too small to give any useful information. Specific labelling of the lipids should be more advantageous for the study of protein-lipid interactions. Some very promising results with fluorine and deuterium labelled lipids have recently appeared in the literature (19, 20).

## 2.2 Micellar and Vesicular Solutions

In liquid crystalline dispersions the motion of the aggregates is very slow (of the order of seconds) due to their size. When such dispersions are broken down in smaller aggregates, either by diluting with water to give micelles or by forming vesicles, a high resolution NMR spectrum is

obtained. All the dipolar interactions have thus averaged to zero due to the fast micelle or vesicle rotation. However, the dipolar couplings will still contribute to the relaxation.

To give a quantitative description of the relaxation it is assumed that the molecular motion can be divided into two independent parts. The first part is the fast local motion of the fatty acyl chains which is assumed to be the same in the micelles or vesicles and the liquid crystalline phase. The second part is the slow motion due to aggregate rotation and/or lateral diffusion. The spin hamiltonian can thus be written (8)

$$H = H_0 + H_f(t) + H_S(t) \quad [2.8]$$

where  $H_0$  is the time independent Zeeman part,  $H_f(t)$  describes the interactions due to fast local motion and  $H_S(t)$  is time dependent with a correlation time  $\tau_r$  determined by aggregate size.

The time dependent part  $H_S(t)$  of the spin hamiltonian in eq. [2.8] will be of the form of eq. [2.6] and the time dependence is solely in the factor  $(3 \cos^2 \beta_{LD}(t) - 1)$  where  $\beta_{LD}$  is the angle between the director and the magnetic field.  $H_S(t)$  can now be written

$$H_S(t) = (3 \cos^2 \beta_{LD}(t) - 1) H'_D / 2 \quad [2.9]$$

with the assumption that  $\tau_r \omega_0 \gg 1$  ( $\omega_0$  is the resonance frequency).

By using conventional relaxation theory (21, 22) the hamiltonian [2.9] leads to a spectrum composed of a number of independent transitions  $\alpha \rightarrow \alpha'$  each giving a lorentzian line with

$$\frac{1}{T_{2\alpha\alpha'}} = (\omega_\alpha - \omega_{\alpha'})^2 \tau_r / 5 \quad [2.10]$$



In addition to the aggregate motion there are fast local motions, which also contribute to  $T_2$  through  $H_f(t)$ . It is crudely assumed that the effect of this motion is uniform and that it can be characterized by a single  $T_2^f$  so that

$$1/T_{2,\text{eff}} = 1/T_2^{\alpha\alpha'} + 1/T_2^f \quad [2.11]$$

The total bandshape is then a superposition of lorentzians with  $T_2$  according to [2.11]. The weight factor for each transition  $\alpha \rightarrow \alpha'$  can be obtained from the bandshape  $L(\omega', 1)$ , eg.

[2.7], for a liquid crystalline sample oriented with  $\cos\beta_{LD} = 1$ . The total bandshape is then

$$L(\omega) = \int_0^\infty \frac{T_{2,\text{eff}}(\omega') L(\omega', 1)}{1 + \omega^2 T_{2,\text{eff}}^2(\omega')} d\omega' \quad [2.13]$$

For proton resonance  $L(\omega', 1)$  has a broad complex shape, due to the interaction of many spins, and according to eq. [2.13]  $L(\omega)$  then consists of superimposed lorentzians with rather different widths. If only a single proton pair interacts with a dipolar coupling,  $L(\omega', 1)$  is a doublet and eq. [2.13] results in a single lorentzian for  $L(\omega)$ .

Equation [2.13] is tested in paper II for a system containing rod-shaped micelles (CTAB-water). The rotational correlation time  $\tau_r$  for these micelles can be varied between  $10^{-7}$  and  $10^{-4}$  seconds by varying the concentration, and thus the whole range of motions, from spherical micelles to the liquid crystalline state, is covered. The lateral diffusion of the molecules around the micelle also can influence the correlation time. With an estimated lateral diffusion coefficient of  $10^{-11} \text{ m}^2/\text{s}$  (12) the lateral diffusion correlation time will be greater than the rotational correlation time and thus can be neglected.

The intensity function  $L(\omega', 1)$  in eq. [2.13] is approximately determined from liquid crystalline phase spectra, in this case a hexagonal phase. The rotational correlation time

$\tau_r$  that comes out from simulations with eq. [2.13] increases rapidly with concentration of the amphiphile and reflects an increase in length of the rod-shaped micelles. Measurements of the anisotropy of the electrical conductivity in flowing solutions (23) give micellar lengths of the same order as in paper II. However, the lengths determined in paper II are upper limits since intermicellar interactions must be of importance at these high concentrations. The great decrease in the contribution to  $T_2$  from fast local motion for the N-methyl protons of cetyltrimethylammonium bromide on increasing concentrations could also be indicative of micelle-micelle interactions.

Another problem when applying eq. [2.13] to micellar or vesicle systems is the question of rate of exchange of amphiphile molecules between aggregates of different size. Rod-shaped micelles are known to be broadly dispersed (24, 25). The mean lifetime of an amphiphile molecule in a micelle can be written (26)

$$\tau = N / (k^+ \cdot \text{c.m.c.}) \quad [2.14]$$

where  $N$  is the aggregation number,  $k^+$  is the association rate constant for micelle formation and c.m.c. is the critical micelle concentration. The association rate constant  $k^+$  is diffusion controlled and is of the order  $10^9$  l/mol s (26, 27). The aggregation number for a 0.35 M cetyltrimethylammonium bromide solution is about 1000 and the c.m.c. is 1 mM. This leads to a lifetime in the micelle of  $10^{-3}$  s.

If the lifetime of an amphiphile molecule in a micelle is shorter than the inverse of the difference in line widths then the fast exchange limit applies. In the above mentioned case the linewidth is of the order of 100 Hz and thus the exchange is fast. However, as the concentration increases, the lifetime for an amphiphile molecule in a micelle will increase according to eq. [2.14]. The linewidth also increases, and the two effects lead to slow exchange conditions. A complicated situation appears when the polydispersity of the micelle is taken into consideration. For the



small micelles fast exchange conditions prevail while the large micelles are in the slow exchange limit.

In paper VII it is shown that viscoelastic solutions of cetyltrimethylammonium salicylate contain large, presumably cylindrical, micelles. For this amphiphile the c.m.c. is an order of magnitude smaller than for CTAB, so in this case slow exchange conditions certainly prevail more.

It is possible to simulate the spectra under slow exchange conditions also. A certain distribution of micelle sizes is assumed (25). By superposition of super-lorentzian curves of different widths and weights according to the distribution, curves of the type in Figure 2.1 are obtained.

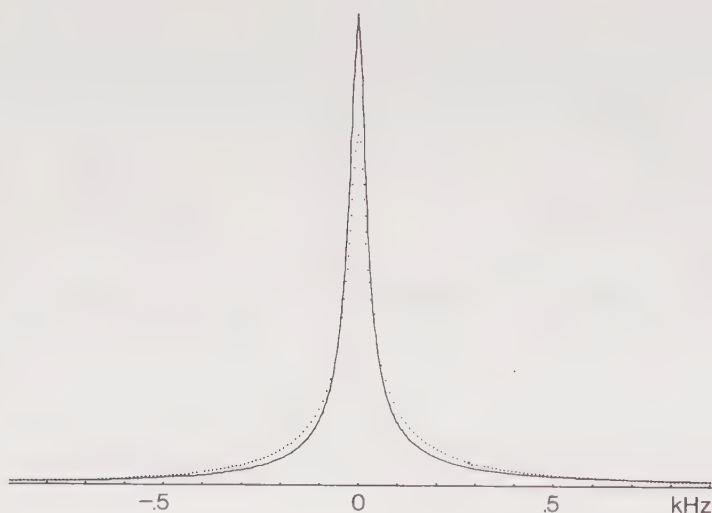


Figure 2.1. Simulated super-lorentzians for slow exchange (full line) and fast exchange (dotted line) of amphiphile molecules between micelles.

For comparison, a super-lorentzian curve for the case of fast exchange with the same mean micellar aggregation number is also shown. As can be seen, the curve for slow exchange has more intensity in the middle and still the same intensity far out in the wings compared to the curve with fast exchange conditions. In the case of slow exchange, very small and very large aggregates will contribute proportionately more to the intensity.

For phospholipid bilayer vesicles the slow exchange conditions certainly apply, but the size distribution is very narrow (25), so a single  $\tau_r$  should be enough to characterize the rotational motion. However, the intensity function  $L(\omega', l)$ , as estimated from randomly ordered liquid crystalline samples, is too broad (see paper I), and an unrealistically short  $\tau_r$  must be used to give a good fit.

The bandshape [2.13] may emerge not only for surfactant aggregates. Other possible systems where eq. [2.13] may be applied are proteins, polymers and adsorbed molecules.



### 3. PHASE EQUILIBRIA AND PHASE STRUCTURE IN LIQUID CRYSTALS

#### 3.1 Deuteron Quadrupole Splittings in Liquid Crystals

The deuteron has the spin quantum number  $I=1$  and thus possesses an electrical quadrupole moment. Through the quadrupole moment the nucleus interacts with electrical field gradients in the surroundings. The nuclear spin hamiltonian may for quadrupole interaction be written (8)

$$H_Q = \beta_Q \sum_q (-1)^q V_{-q}^{(2)} A_q^{(2)} \quad [3.1]$$

where the  $V_q^{(2)}$ 's are irreducible components of the electrical field gradient tensor and the  $A_q^{(2)}$ 's the standard components of a spin tensor operator (cf. eq. [2.1]).  $\beta_Q$  is given by  $eQ/2I(2I - 1)\hbar$  where  $eQ$  is the quadrupole moment.

In an isotropic liquid, the mean value of  $H_Q$  is zero, and the quadrupole interaction contributes only to relaxation. In an anisotropic medium, for example a lamellar mesophase, the mean value of  $H_Q$  is no longer zero and a quadrupole splitting appears in the NMR spectrum. As mentioned in section 2.1, it is most convenient to express the spin operators in the laboratory-fixed coordinate system (L), while the electrical field gradients are expressed in the molecular coordinate system (M) fixed at the nucleus. Using the same procedure for coordinate transformation as in section 2.1  $H_Q$  can be written:

$$H_Q = \beta_Q \sum_{qq'q''} (-1)^q V_{-q}^{(2)M} A_{q''}^{(2)L} D_{q'q}^{(2)}(\Omega_{DM}) D_{q''q'}^{(2)}(\Omega_{LD}) \quad [3.2]$$

In eq. [3.2] only  $D_{q'q}^{(2)}(\Omega_{DM})$  varies with molecular motion.

If there is a threefold or higher symmetry around the director axis, then the mean value  $D_{q'q}^{(2)}(\Omega_{DM})$  is zero for  $q' \neq 0$ , and for the usual case, where the quadrupole coupling is weak compared to the Zeeman term ( $q'' = 0$ ), this results in

an NMR spectrum with a doublet symmetry with respect to the Larmor frequency and with a splitting:

$$\Delta(\beta_{LD}) = \left| \nu_Q S (3\cos^2\beta_{LD} - 1) \right| \quad [3.3]$$

where  $\beta_{LD}$  is the angle between the magnetic field and the director, the quadrupole coupling constant  $\nu_Q = 3/2 \beta_Q$  and the order parameter

$$S = 1/2 \{ (3\cos^2\beta_{DM} - 1) + \eta (\sin^2\beta_{DM} \cos 2\gamma_{DM}) \} \quad [3.4]$$

$\eta$  is the molecular asymmetry parameter.

For systems with lower symmetry some  $\overline{D_{q'q}^{(2)}(\Omega_{DM})} \neq 0$  and the  $^2H$  NMR spectrum will have the same appearance as for a static system with the asymmetry parameter  $\eta \neq 0$  (11, 31).

For a powder sample where all values of  $\cos\beta_{LD}$  are equally probable the NMR spectrum consists of a broad absorption curve with two marked peaks separated by  $\Delta = \left| \nu_Q S \right|$ .

### 3.2 Model Membrane Systems

A common characteristic of all membrane phospholipids is the existence of a phase transition which involves a disordering of the hydrocarbon chains in the interior of the membrane (28-30). Following the nomenclature of Tardieu (32) the ordered state is denoted  $L\beta$  or  $L\beta'$  and the disordered state  $L\alpha$  (see Figure 3.1).

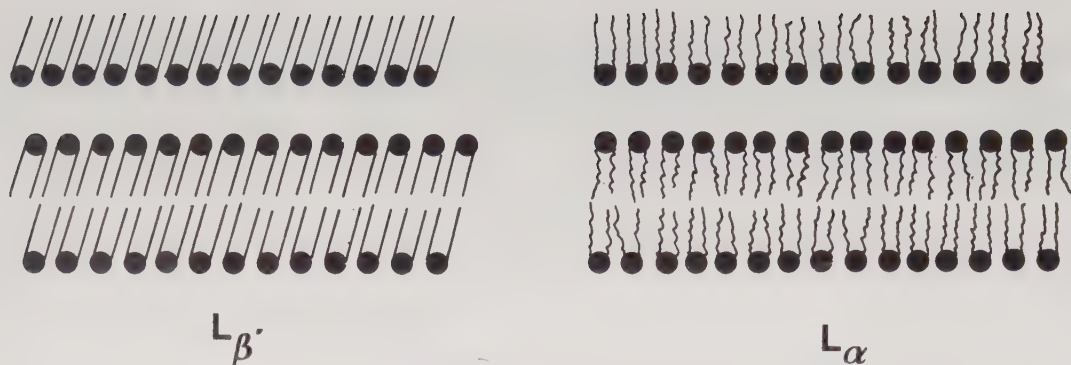


Figure 3.1. Schematic representation of the  $L_{\beta'}$  and  $L_{\alpha}$  structures.

For certain water contents and temperatures, two water-deuteron splittings are observed (paper III) in the system dipalmitoyl phosphatidyl choline-water, which can be explained by the presence of two different phases, in the case of phosphatidyl choline, an  $L_{\alpha}$  and an  $L_{\beta'}$  phase. The ratio between the two phases can easily be obtained by simulation of the spectrum. The existence of two independent splittings implies that the lifetime of a water molecule in respective phase is at least  $10^{-4}$  s, or, put in another way, if the diffusion coefficient for water is  $10^{-10} \text{ m}^2/\text{s}$  (33), the size of the phase is at least of the order 1  $\mu\text{m}$ .

From this argument it also follows that if  $L_{\alpha}$  and  $L_{\beta'}$  phases coexist within the same bilayer (lateral phase separation),  $L_{\alpha}$  regions must be stacked above  $L_{\alpha}$  regions, and  $L_{\beta'}$  regions above  $L_{\beta'}$  regions, for many bilayers. However, Gottlieb and Eanes (34) found such a long range ordering improbable, and instead suggested that the two states exist in separate phases.

For lipid mixtures there is direct evidence that different phases coexist within the same bilayer (35-37) with domain sizes of several  $\mu\text{m}$ . We have not been able to observe separate water-deuteron splittings from different phases in mixtures



of dimyristoyl- and distearoyl phosphatidyl choline (38) and a possible explanation is that there is no long range ordering of the phase domains between the bilayers.

Typical for the phosphatidyl cholines is the pretransition observed 3-12 degrees below the main transition depending on hydrocarbon chain length. Several structures for the phase between the pre-transition and the main transition have been put forward. Tardieu et al. (32) and Janiak et al. (39) proposed a rippled structure ( $P\beta'$ , Figure 3.2) while Rand et al, (40) suggested an  $L\beta$  phase (Figure 3.2).

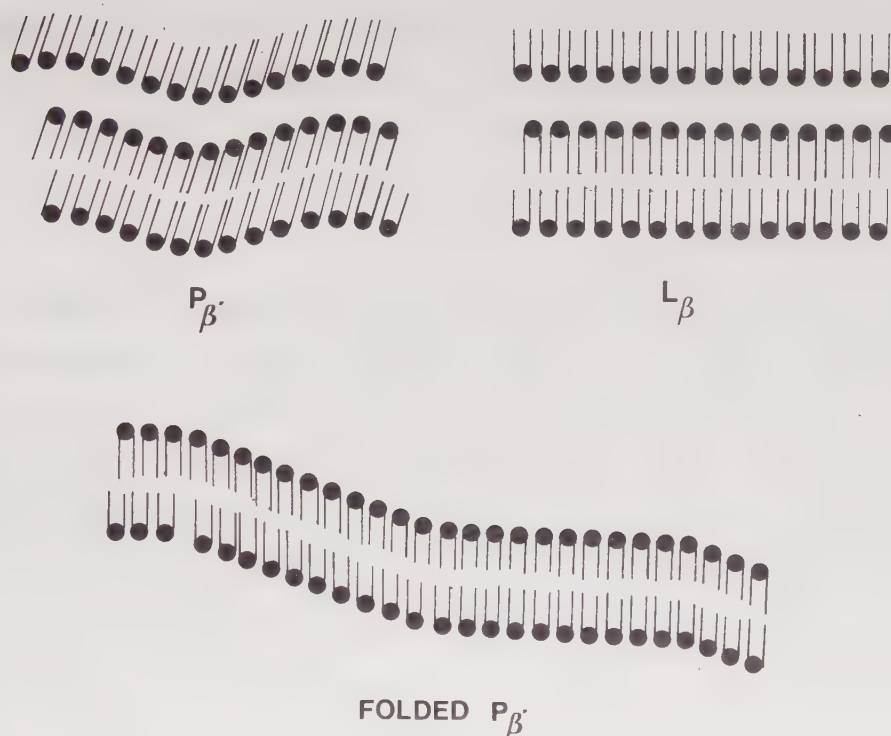


Figure 3.2. Schematic representation of the  $P\beta'$ ,  $L\beta$  and folded bilayer structures.

Recently Larsson (41) has modified the model by Rand et al. (40) by suggesting a folded lamellar structure with lamellar units with vertical chains alternating with lamellar units with tilted chains (Figure 3.2). An  $L\beta$  type phase is also unlikely since observations by electron microscopy (42)

show periodic variations with an order of 150 Å, the same value as the ripple periodicity observed by X-ray techniques (39).

In the region between the pre-transition and the main transition a very narrow water deuteron splitting is observed. Both structures suggested above are compatible with this narrow splitting. The  $P\beta'$  phase or the folded lamellar structure have lower than threefold symmetry around the director axis, which should result in an overall asymmetry parameter  $\eta \neq 0$  and thus a decrease in the splitting. Another mechanism that could cause smaller splittings is if the water molecules can have several different orientations with respect to the bilayer plane during the diffusion along the lamellae. The diffusion is so fast that it is possible for the  $P\beta'$  phase, but it is more difficult to imagine in the case of a folded lamellar structure.

From a systematic investigation of the water deuteron splittings in the dipalmitoyl phosphatidyl choline water system and with the help of the phase rule it is possible to construct the phase diagram, as shown in paper III. It is characteristic that the phase between the pre-transition and the main transition has very specific hydration requirements for its existence. The formation of a structural water matrix around the head group thus appears to be a requirement for the formation of this phase. Also the choline head group seems to be essential. Dipalmitoyl phosphatidyl ethanolamine and its N-mono and N,N-dimethyl analogues do not exhibit a pre-transition (43).

### 3.3 Acholeplasma laidlawii Membrane Lipid Systems

The membranes of the microorganism Acholeplasma laidlawii contain as major polar lipids the uncharged glycolipids monoglucosyldiglyceride (MGDG) and diglucosyldiglyceride (DGDG) (Figure 3.3).





As the maximum hydration of the two lipids is about the same, the binding conditions for water must be quite different in MGDG compared to DGDG. The low water binding of MGDG also suggests that the hexagonal structure is of the reversed type (Figure 3.4).

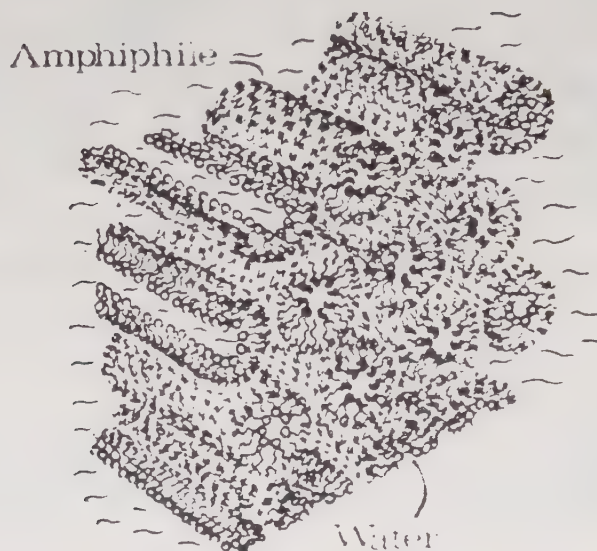


Figure 3.4. The structure of the reversed hexagonal mesophase.

The hydroxyl groups of the glycolipids are the likely water binding sites. Since MGDG takes up the same amount of water as does DGDG, several of the more abundant hydroxyl groups of DGDG must be shielded from water binding. A possible configuration of the polar head group is then with the sugar residues parallel to the bilayer plane. Divalent ions such as calcium or magnesium have pronounced effect on the temperature dependence of the water-deuteron splittings for DGDG. A hysteresis effect is observed as the splittings normally occurring above the transition temperature do not disappear upon cooling until 15-20° below the normal transition. On heating again, the splittings again appear at the normal transition temperature. Below the transition, water binding is very limited as only an isotropic peak is observed in the  $^2\text{H}$  NMR spectrum. The ions are probably concentrated in regions with this "isotropic" water. However, above the

transition, water interacts with the glycolipid, and the divalent ions can probably also interact with the sugar groups. (It is known that divalent ions can interact with sugars (44)). This will form a new liquid crystalline state with another transition temperature.

### 3.4 The System Lecithin-Sodium Cholate-Water

The system lecithin-bile salt-water is important biologically because the primary constituents, lecithin and bile salt, are the solubilizing system of bile for insoluble nonswelling amphiphiles, in particular free cholesterol, which occurs in bile in high concentrations.

Especially the system lecithin-sodium cholate-water has been thoroughly studied by Small and coworkers (45-48) and is shown in Figure 3.5.

The most remarkable feature is the structure of the hexagonal phase, where stacked bilayer discs of lecithin coated with sodium cholate molecules build up the cylinders. This type of cylinder differs in structure from those of other amphiphiles (Figure 1.4a) in that there is no continuous hydrocarbon region along the cylinder. In the lamellar phase, sodium cholate probably exists as dimers incorporated in the lecithin bilayer, due to intermolecular hydrogen binding between the hydroxyl groups.

In paper V this system is studied by different NMR methods and also X-ray diffraction in order to extract information about the molecular organization of the different phases.

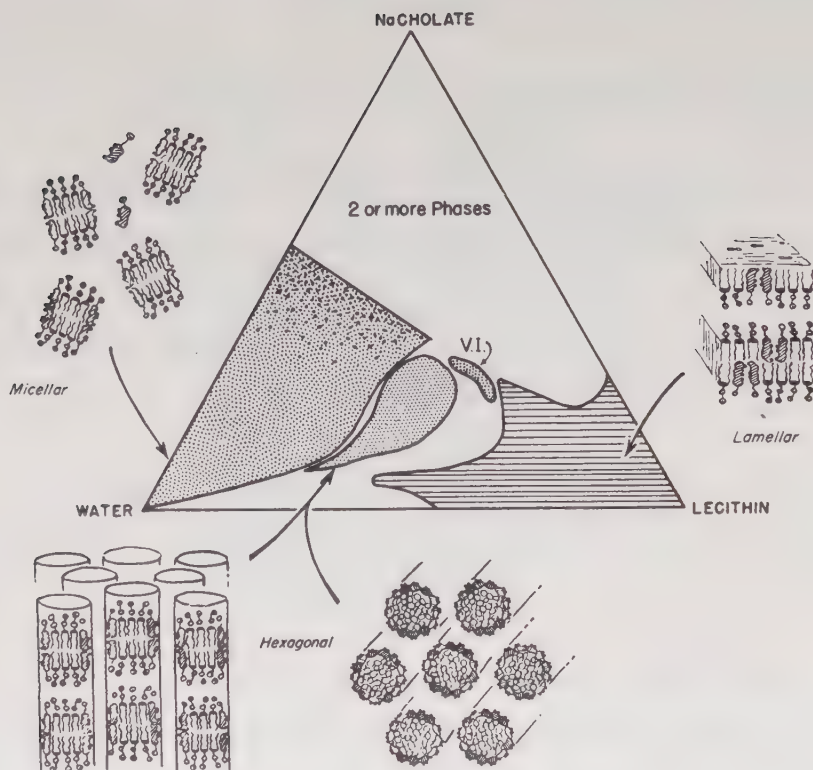


Figure 3.5. The ternary system lecithin-sodium cholate-water.

The first striking difference between the lecithin-sodium cholate-water system and simple amphiphile-water systems is the similarity of molecular order in the lamellar and the hexagonal phase. In simple soap-water systems the order parameter generally differs by a factor of two between the lamellar and hexagonal phases (cf. section 3.3). This similarity in order is in full accordance with the models proposed by Small, since the hexagonal phase can be considered as lamellae chopped in small discs.

The molecular order in both the lamellar and the hexagonal phase is very sensitive to the ratio between lecithin and sodium cholate. As the cholate content increases, the order



parameter for the alkyl chains of lecithin decreases drastically. Also the water molecules sense a decrease in ordering with higher cholate contents. These trends are just the opposite to the influence of cholesterol on lecithin bilayers (11, 49). One would expect that sodium cholate dimers incorporated in the lecithin bilayers have the same effect as cholesterol if the dimers are arranged in the same way as cholesterol in the bilayer.

To account for the disordering effect of sodium cholate it is suggested that cholate molecules are solubilized more randomly in the bilayer, as shown in Figure 3.6.

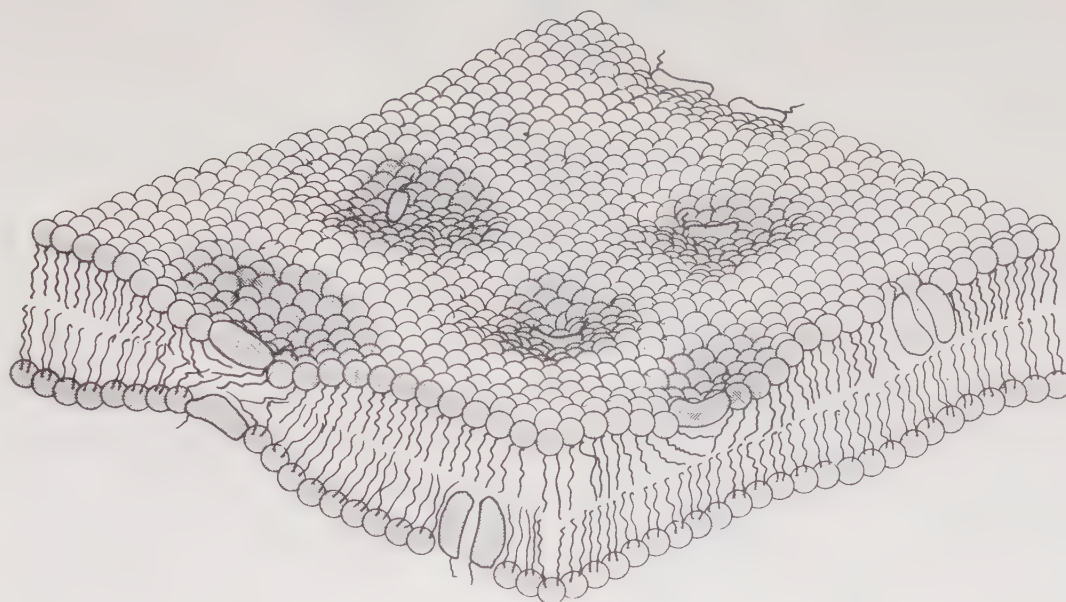


Figure 3.6. Suggested structure of the lamellar phase in the system lecithin-sodium cholate-water.

Here the cholate molecules, besides existing as dimers, also can lie flat in the bilayer surface with the hydrophilic side facing towards the water layer. This arrangement introduces a non-planarity in the bilayer which decreases the order parameter further. The same organization should of course apply to the hexagonal phase.

So far all NMR data are compatible with Small's model for the hexagonal phase, consisting of cylinders of stacked bilayer discs. X-ray diffraction data is not of the degree of clarity obtained from other hexagonal amphiphile systems, which can be due to extra symmetries from the periodic stacking of bilayer discs. However, NMR diffusion measurements indicate that the rods in the hexagonal phase have a continuous lipid region over long distances, which is in contradiction to Small's model. To account for these observations it is suggested that the hexagonal phase consists of more or less flat rods, as shown in Figure 3.7. The X-ray data is equally valid for this model.

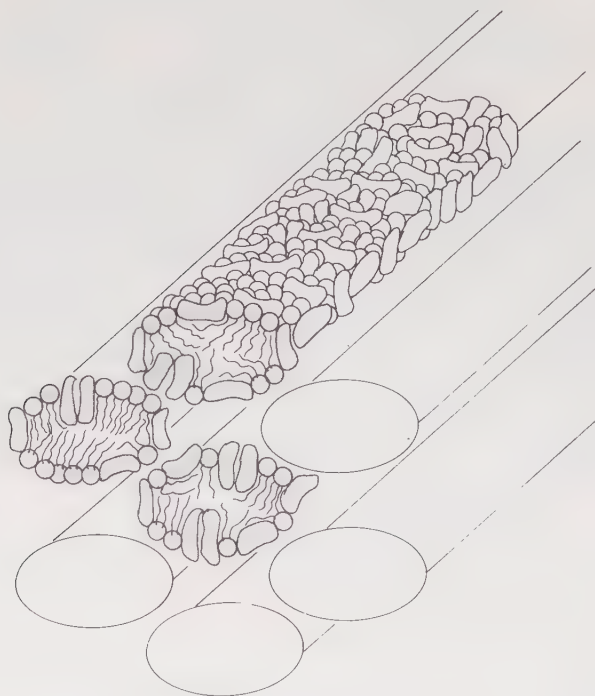


Figure 3.7. Suggested structure of the hexagonal phase in the system lecithin-sodium cholate-water.

At lower water contents the hexagonal phase passes into a cubic phase (Figure 3.5). Also in this phase, NMR diffusion measurements indicate continuous amphiphile aggregates (50). A structure that is compatible with NMR data and the molecular arrangement of cholate in the lecithin bilayer is Neovius' cubic structure in Figure 3.8.

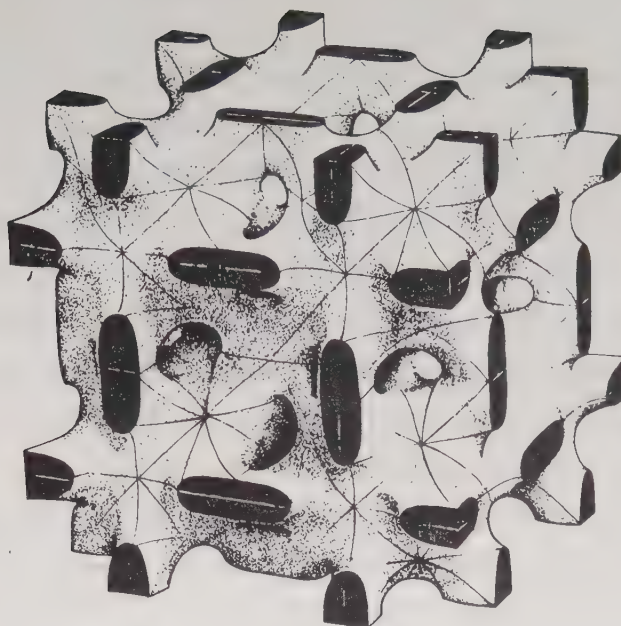


Figure 3.8. The Neovius' cubic structure.

The NMR diffusion measurements are strong evidence for continuous hydrocarbon regions in the hexagonal and cubic phases, which is in opposition to the model suggested by Small. The interpretation of X-ray data for the hexagonal phase is not obvious, but it is possible that additional symmetries besides the hexagonal exist. In the isotropic phase, the micelles stay relatively small right up to the phase boundary, which is different from other system, where a hexagonal phase is formed. On increasing the concentration of amphiphile in the micellar phase, the micelles usually grow in size with increasing concentration (cf. section 1.3).



#### 4. COUNTERION SPECIFICITY AND VISCOELASTICITY IN MICELLAR SYSTEMS

In paper II it was shown that the proton NMR bandshape changed drastically when going from spherical micelles to large rod-shaped micelles. In the system cetyltrimethylammonium bromide (CTAB)-water this transformation takes place at a concentration of about 10 %. This transition is also observed by  $^{81}\text{Br}$  NMR relaxation (51). However, for cetyltrimethylammonium chloride micelles there is no such transition (51).

The reason for this difference between chloride and bromide ions can be found in the higher polarizability of the bromide ion. The bromide ions can thus be located closer to a phase boundary than chloride ions (52), and the electrostatic repulsion between the head groups will be smaller, resulting in a closer packing. As mentioned in section 1.1 a decrease in head group area, without changing the hydrocarbon chain volume, will favour a rod-shaped aggregate.

Partial substitution of chloride ions by bromide ions or vice versa does not affect the relaxation rates of respective ions (51, paper VI). A conclusion that immediately suggests itself is that the solutions contain a mixture of spherical and cylindrical micelles. The chloride ions preferentially interact with spherical micelles, while bromide ions interact with cylindrical micelles.

Dilute solutions of cetyltrimethylammonium salts and additives sometimes behave extraordinary. As an example, when 1-methyl-naphthalene is added to a 1% solution of CTAB, the solution becomes viscoelastic. This property is easily seen by swirling the solution. After swirling is stopped air bubbles trapped in the solution appear to recoil before becoming stationary.

In elastic material as rubber or gelatine the molecular arrangement is an interlinking structure of polymer chains. It is more difficult to imagine what type of structure causes viscoelasticity in dilute amphiphile solutions.

In paper VI it is shown that solubilization of 1-methylnaphthalene occurs toward the polar part of the CTAB micelles. In paper VII an even more spectacular viscoelastic amphiphile system is described, namely the two-component system cetyltrimethylammonium salicylate (o-hydroxybenzoate), which shows viscoelastic properties down to 0.01%. The benzene ring of the salicylate anion is solubilized in the micelle to some extent. As mentioned before, a large ratio between the polar head group area and the hydrocarbon chain volume (at a given length of the hydrocarbon chain) favours a spherical micelle, while a smaller ratio favours a cylindrical form. 1-methylnaphthalene will not have so large effect on the polar head group area, but the hydrocarbon chain volume is expected to increase, which will favour a cylindrical micelle shape. At higher solubilizate/amphiphile ratios the 1-methylnaphthalene molecule solubilizes further into the micelle with an increase of the effective length of the amphiphile as result. The micelle then may resume the globular shape. Above a molar ratio 1-methylnaphthalene/CTAB of 0.7 this system loses its viscoelastic properties. For the system cetyltrimethylammonium salicylate it is probably the screening effect of the salicylate anion that is most important for the micelle shape. The solubilization of the benzene ring will increase this effect. The very similar system CTA-p-hydroxybenzoate show no viscoelastic properties. In this case the solubilization of the aromatic ring will force the hydroxyl group into an unfavourable apolar surrounding. The screening effect of the p-hydroxybenzoate anion will be less and the micellar shape will remain spherical.

NMR and linear dichroism data in paper VII suggest that a necessary condition for viscoelasticity in amphiphile solu-

tions is aggregates larger than the minimum spherical micelle. The mechanism that leads to a viscoelastic behaviour in dilute solutions of rod-shaped micelles is more difficult to elucidate. The network structure suggested (53) seems improbable. Instead it is proposed that the micellar aggregates are ordered over macroscopic distances, forming a periodical structure. If the resultant potential between two aggregates, due to electrostatic and dispersion interactions, is located in the region of positive energy values (Figure 4.1), this will result in the fixation of the aggregates and the appearance of elastic properties (54).

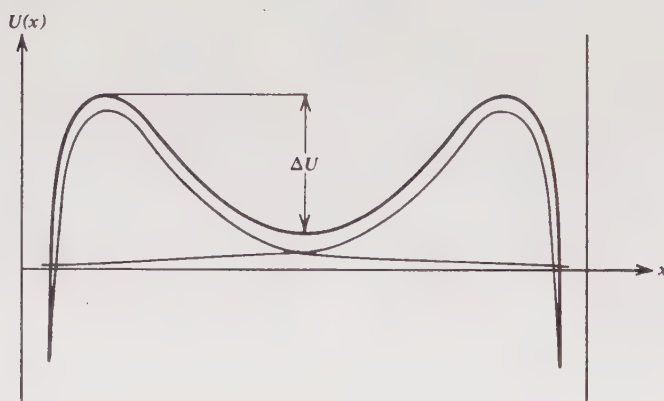


Figure 4.1. The resultant potential curve between two particles under restricted volume conditions.



## 5. ACKNOWLEDGEMENTS

Håkan, Göran and Gösta, for your guidance that made this thesis possible. Your knowledge, enthusiasm and friendship were invaluable;

Sture, for encouraging an open-minded atmosphere at the department and providing stimulating working conditions with the best NMR equipment;

Björn, for frequently trying to put some pep into me, although my response was not always very impressive;

Torbjörn, Hans and Lennart, for patiently teaching the secrets of the spectrometers, especially the art of where to pull and kick;

Annika, Karin and Gunilla, for your help with preparing various substances;

Gun-Britt, Eva, Bodil, David and Dennis, for typing and linguistic corrections of this and various other papers;

Krister, Åke, Lennart, Signe and everyone else at the department:

T H A N K     Y O U

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THE LIQUID CRYSTALLINE PHASES OF THE LECITHIN-SODIUM CHOLATE  
WATER SYSTEM. MOLECULAR ORGANIZATION STUDIED BY NMR AND LOW  
ANGLE X-RAY DIFFRACTION METHODS

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## ABSTRACT

The liquid crystalline phases of the system lecithin-sodium cholate-water are investigated, using different NMR methods and X-ray diffraction.

$^1\text{H}$  and  $^2\text{H}$  NMR spectroscopy shows that the molecular ordering is very similar in the so called hexagonal and the lamellar phases in contrast to what is found for ordinary soap systems. It is found that the molecular order decreases strongly with increasing cholate concentration.

The X-ray diffraction picture for the so called hexagonal phase shows reflexions that do not unambiguously fit the hexagonal structure.

Lipid diffusion coefficients for the cubic and the "hexagonal" phases are of the same order of magnitude as those from the lamellar phase, and it is concluded that the cubic and the "hexagonal" phases consist of continuous amphiphile aggregates. This is not compatible with the structure previously proposed for the "hexagonal" phase, where micelles are stacked to form long rods. Previous models of the different phases have therefore been modified according to the present observations.



## INTRODUCTION

The intestinal absorption of fats cannot be described in molecular terms without a thorough knowledge of the structure and properties of the system phospholipid-bile salt-water. The physico-chemical properties of bile salts in various binary, ternary or quaternary systems have been extensively studied by Small and coworkers, and most of their results are summarized in a review by Small<sup>1</sup>. Bile salts are surface active compounds but have several properties that differ from those of typical amphiphiles as e.g. soaps. Thus, the cooperative aggregation of bile salts seldom leads to the formation of micelles<sup>1,2</sup>. Only the deoxycholate having two hydroxylic groups can form aggregates of substantial size and which possess micelle-like properties, while for the other bile salts only oligomeric aggregates are found<sup>1,3</sup>. Furthermore, a mixture of bile salts and water

has never been observed to form lyotropic liquid crystalline phases. As was pointed out by Small<sup>1</sup> these differences between the bile salts and typical amphiphiles are physiologically highly relevant. For example one of the unique properties of the bile salts are their capacity to break up lecithin bilayers by forming soluble mixed micelles<sup>1</sup>. It has also been shown that a lecithin bilayer cannot incorporate more than one cholate molecule per two lecithin molecules<sup>4,5</sup>. For another swelling amphiphile, mono-octanoin, any proportion between a typical amphiphile like octyl-ammonium chloride and the monoglyceride can form a lamellar mesophase<sup>6</sup>. Therefore it can be assumed that also lamellae consisting of a mixture of lecithin and a soap are stable over a much larger range of mixing than the lecithin-cholate lamellae.

The stability and formation of liquid crystalline and micellar aggregates with different shapes have been discussed previously<sup>7,8</sup>. For example it was shown that the transformation of a lamellar liquid crystalline phase into an isotropic aqueous solution requires that aggregates growing in one dimension only should be more stable than the two-dimensional lamellar structure. As an example we can take the lamellar phase of soap-alcohol systems, which can in fact be made soluble by addition of soap<sup>8,9</sup>. From the result in refs. 7 and 8 it is also logical that a liquid crystalline phase with rod-like aggregates (i.e. micelles growing in one dimension) is formed when decreasing the water content of a lecithin-bile salt aqueous solution with mixed micelles. Furthermore, as for micellar solutions of soaps<sup>8,10</sup> one also expects for the lecithin-bile salt system, a structural similarity between the micellar aggregates and the aggregates of infinite extension that build up the liquid crystalline phase formed at lower water concentration<sup>4</sup>.

The ternary phase diagram of egg yolk lecithin-sodium cholate-water has been determined by Small and coworkers<sup>4,5</sup> (Fig. 1). As can be seen from this diagram a lamellar phase occurs at high lecithin-bile salt ratios and at low water content. In the "hexagonal" phase in the middle of the triangle diagram the minimum amount of bile salt necessary for its formation is again one bile salt molecule to two lecithin molecules. A third mesophase also exists, namely a so-called viscous isotropic phase at high bile salt to lecithin ratios and at low water content. Finally, we have isotropic micellar solution at high water concentrations.

The visual appearance of the different phases in the diagram and the similarities between this phase diagram and those found for soap-

-alcohol-water systems<sup>9</sup> led Small<sup>1</sup> and coworkers to conclude that the molecular packing was similar in the different systems. However, important differences soon became clear to these workers, particular concerning the structure of the "hexagonal" phase. From the results of various physical techniques (e.g. light scattering, NMR and x-ray diffraction) Small<sup>1</sup> suggested that the mixed micelles are disc-shaped lecithin lamellae with the cholate anions arranged around the circumference of the disc having their hydrophilic side exposed to the water (cf. Fig. 1). The "hexagonal" phase then consists of a stack of such discs with a small amount of intervening water (cf. Fig. 1). In a recent study<sup>11</sup> Shankland has shown that this structure model is consistent with the x-ray diffraction data obtained for a large number of samples in the "hexagonal" phase. Mazer et al.<sup>12</sup> also showed that laser light scattering data for the micellar phase indicated disc-like aggregates although the original model shown in Fig. 1 had to be slightly modified to fit the experimental data. A second recent report<sup>13</sup> on laser light scattering of the mixed bile salt-lecithin micellar system<sup>13</sup> indicated that the addition of lecithin did not alter the micellar size. This finding is contradictory to the interpretation by Mazer et al.<sup>12</sup>.

Very few if any unambiguous experimental results have been reported on the molecular packing and the molecular order in the amphiphilic aggregates. NMR and other spectroscopic techniques can give information which complements the structural data obtained by x-ray diffraction studies. The attention is focused on the molecules themselves and not on the gross dimensions of the aggregates they form.

Measurements of the translational diffusion of lecithin by a pulsed NMR technique showed that the lipid diffusion coefficient for the



viscous isotropic phase in the system shown in Fig. 1 was of the same order of magnitude as that obtained for the lipid diffusion in the lamellar phase<sup>14,15</sup>. These findings are inconsistent with a model, where the amphiphilic aggregates of the isotropic mesophase consist of rod-like aggregates with the same structure as suggested for the hexagonal phase in Fig. 1. Instead the aggregates of this phase have to be continuous with respect to the lipid regions over large distances. Structural models of this kind have also been suggested previously by Luzzati<sup>16</sup>.

The behaviour of the lecithin diffusion in cubic and lamellar mesophases is analogous to what was found for soap-water systems<sup>15,17</sup>. Therefore we suggested that the molecular packing is similar to that in the corresponding soap aggregate.

In order to extract information about the molecular organization in the different liquid crystalline phases, we have extended our studies of the lecithin-cholesterol system to comprise the lamellar, the cubic and the "hexagonal" phases. For this purpose we have used a number of magnetic resonance methods and to a lesser extent also x-ray diffraction. First, we will consider qualitatively the variation of the molecular order between different phases. Then follows a quantitative discussion of the ordering of lecithin and water molecules. The effects of macroscopical alignment are considered and lipid translational diffusion in all mesophases results are discussed in connection with different structural models for the three mesophases in question.

## MATERIAL AND METHODS

Egg-yolk lecithin was prepared from freshly extracted egg-yolk lipids by column chromatography on aluminum followed by chromatography on silicic acid. Synthetic lecithins were obtained by acylation of sn-glycero-3-phosphocholine with the fatty acid anhydrides<sup>18</sup>. These and sn-glycero-3-phosphocholine were prepared by established procedures<sup>19,20</sup>. The synthetic lecithins were purified by column chromatography on silicic acid. All lecithin preparations were subjected to analysis by thin-layer and gas chromatography for the assessment of purity. Serial dilutions of each sample over a hundred-fold concentration range were applied to silica gel thin-layer plates run in different solvent systems. After development the plates were sprayed with dichlorfluorescein and viewed under UV-light, followed by spraying with ninhydrin and finally with a phospholipid specific spray reagent. Only lecithins with a purity  $\geq 99\%$  were used.  $\omega$ -d<sub>3</sub> dodecanoic acid and 8,8-d<sub>2</sub> tetradecanoic acid were prepared by Kolbe electrolysis as described by Dinh-Nguyen<sup>21</sup>. The fatty acids were characterized by their melting points and mass spectra. Cholic acid was obtained from BDH, Poole, England and the sodium salt was prepared by neutralizing an alcoholic solution of cholic acid with an equimolar amount of sodiummethoxide, then boiling of the alcohol until precipitation started. The sodium cholate was dried in vacuum. The water was double-distilled in an all-quartz apparatus. Heavy water purchased from Ciba-Geigy, Switzerland, was used.

Samples were prepared in two different ways. In one method lecithin and sodium-cholate were weighed into an ampoule and the mixture was dissolved in methanol. Then the solvent was evaporated in vacuum. A given quantity of water was added with a microsyringe and the contents of the ampoule were mixed. The ampoule was then sealed

and the mixture allowed to equilibrate for several days. In the other method sodium cholate was dissolved in water and known amounts of this solution and lecithin were mixed. No differences between these two methods of sample preparation could be observed by x-ray diffraction or NMR spectroscopy. Macroscopically aligned phases were prepared by pressing a sample between microscope cover glasses.

$^1\text{H}$  NMR measurements were performed at 100.0 MHz with a Varian XL-100-15 NMR spectrometer operating in the continuous wave mode. The same spectrometer was used to record  $^2\text{H}$  FT NMR spectra at 15.4 MHz. Some  $^2\text{H}$  NMR measurements were performed with a modified Bruker BKR 322 s spectrometer at 13.8 MHz using the quadrupolar echo technique<sup>22</sup>. The diffusion measurements were performed using pulsed NMR with pulsed magnetic field gradients as described previously<sup>15,24</sup>.

The specimens were examined by polarizing microscopy and studied by x-ray low-angle diffraction using a pinhole type of camera where the samples were enclosed in flame-sealed thin-walled capillary tubes. The technique has been previously described<sup>23</sup>.

## RESULTS

### A. Proton magnetic resonance spectra

Some ten years ago it was shown by Lawson and Flautt<sup>25</sup> that the  $^1\text{H}$  magnetic resonance spectra for the alkyl chains of the lipids in unoriented liquid crystalline samples exhibit a characteristic shape called "super-Lorentzian" having a narrow central peak and unusually broad wings. In the anisotropic mesophase the intramolecular dipole-dipole interactions between the proton spins do not average to zero. This is generally true except for a part of the samples namely for the "microcrystallites" where the orientation



of the symmetry axis of the liquid crystalline phase makes an angle equal to  $54.7^\circ$  relative to the applied magnetic field. Here the dipole-dipole couplings vanish. This effect causes the appearance of the relatively weak central signal in the spectrum, while the total width of the peak is determined by the size of the dipole-dipole couplings<sup>26-28</sup>.

Both lecithin and cholate contain a multitude of protons and therefore the  $^1\text{H}$  NMR experiments give no detailed information about the molecular order. However, the width of the peaks can give an approximate measure of the molecular packing of the carbon chains of the lecithin at different compositions. Fig. 2 shows  $^1\text{H}$  NMR spectra for two samples from the lamellar and one from the "hexagonal" phase. The spectra given in Fig. 2b and c are taken at constant ratio between lecithin and cholate so that only the water content has been changed to produce the transition between the lamellar (Fig. 2b) and the "hexagonal" (Fig. 2c) phases. It is surprising that the spectra obtained for the two different phase structures are essentially the same. This finding is in contrast to what has been observed for soap systems<sup>29</sup>, where the lamellar phase has a four times larger second moment than the hexagonal phase. It should be noted that we have not been able to reproduce the high resolution peak previously reported<sup>1</sup> for the "hexagonal" phase (cf. Fig. 2c). Theoretically such an observation is not expected either, so we have to conclude that this high resolution peak is due to a contamination from an isotropic phase, probably a cubic mesophase or micellar solution (see the phase diagram in Fig. 1). This spectrum has been put forward to be a strong support for the models of the structures depicted in Fig. 1. The spectrum in Fig 2c is in fact much more compatible with these structures, although, as will be shown below, also other models are possible.

A comparison between the widths of the peaks in Fig. 2a and b for the lamellar phase at different cholate concentrations reveals that there is a decrease in the width at higher cholate content. This means that the molecular order of the alkyl chains decreases as the cholate content is increased.

#### B. Deuterium magnetic resonance

The deuterium nucleus has a quadrupole moment giving rise to quadrupole splittings in the  $^2\text{H}$  nuclear magnetic resonance spectra for anisotropic mesophases, such as the lamellar and hexagonal phases<sup>30</sup>. Studies of specific deuterated alkyl chains of the lecithin molecules can give information about the average orientation of the acyl chain segments of these molecules in the amphiphilic aggregate. The average orientation is characterized by an order parameter  $S_{\text{mol}}$  that can be calculated from the measured quadrupole splitting  $\Delta$ . The relation between  $S_{\text{mol}}$  and  $\Delta$  for a  $\omega$ -methyl group is given by<sup>30</sup>:

$$|S_{\text{mol}}| = \frac{8}{3} \frac{\Delta}{\chi} \quad \text{where } \chi \text{ is the quadrupole coupling constant}$$

which has been determined<sup>31</sup> to be 170 KHz for a paraffinic C-D bond.

Fig. 3 shows how  $S_{\text{mol}}$  calculated from the deuteron splitting of the  $\omega$ -methyl groups of dilauroyllecithin varies with the ratio between lecithin and cholate. The two terminal methyl groups are not chemically equivalent and therefore their quadrupole splittings are different as is shown in Fig. 3. It can be inferred from Fig. 3 that  $S_{\text{mol}}$  decreases with increasing cholate content but there is no discontinuous change of the order parameter between the lamellar and the "hexagonal" phases (cf. the  $^1\text{H}$  NMR experiments discussed above). The deuterium quadrupole splitting,  $\Delta$ , determined for the  $\text{C}_8$ -positions of dimyristoyl lecithin are given in Table I for three samples of the

lecithin-cholesterol water system. Again a given lecithin/cholesterol ratio leads to the same value of  $S_{\text{mol}}$  for the lamellar and "hexagonal" phases. Note the three times larger splitting obtained for a lamellar phase without cholesterol.

Quadrupole splittings from water deuterons can be utilized to study the orientation of the water at the amphiphilic surfaces<sup>32,33</sup>. In a previous work it was shown<sup>33</sup> that the water orientation decreases with increasing cholesterol content and that there was not discontinuity in the splitting at the transition between the lamellar and "hexagonal" phases. This investigation has been extended here by using a high resolution spectrometer. A typical spectrum is shown in Fig. 4, where it can be seen that the effective asymmetry parameter<sup>30</sup> is close to zero. This result indicates that the average phase structure has a threefold or higher axis of symmetry<sup>34</sup>. The water splittings obtained are summarized in Table II. It can be observed from this table that at lower water contents the water orientation strongly depends on the ratio between lecithin and cholesterol.

### C. Macroscopically aligned samples between glass plates

Usually, nonoriented liquid crystalline samples were used in the studies described above. For these samples the axis of symmetry of the mesophase is randomly oriented. It is, however, possible to prepare a macroscopically aligned sample by pressing the samples between glass plates<sup>35,36</sup>. The orientation of the symmetry axis with respect to the glass will then depend on the structure of the phase itself. Lamellar phases have been found to align with the bilayers parallel to the plates<sup>35,36</sup>. It has also been shown previously that when the normal to the plates makes an angle equal to  $54.7^\circ$  (the so called magic angle) relative to the applied magnetic field the dipolar



couplings vanish. Thus the proton spectrum will be substantially narrowed at the magic angle as is illustrated in Fig. 5.

For the hexagonal phase the symmetry axis is expected to orient parallel with the plates but randomly oriented in the plane of the plates. Here the orientation dependence of the spectrum is substantially weaker. Theoretically the maximal intensity should be obtained<sup>32</sup> when the normal to the plates makes  $35.3^\circ$  with respect to the magnetic field.

The water deuteron quadrupole splittings have also been studied at different angles for this oriented "hexagonal" phase as is shown in Fig. 6. These spectra show more clearly that the "hexagonal" phase orients with the symmetry axis cylindrically distributed<sup>32,34</sup> around the normal to the glass plates.

#### D. Diffusion studies

The translational diffusion coefficients for the cubic<sup>14</sup> and lamellar<sup>15,27</sup> phases have previously been determined with pulsed NMR methods. Here we have performed some new measurements of the diffusion coefficient of egg yolk lecithin at different cholate concentration for the lamellar phase. The same technique as described previously<sup>15</sup> with macroscopically aligned samples were used. Although hexagonal phases can be oriented between glass plates, such samples cannot, in most cases be used for diffusion measurements. This is due to a much too low intensity of the microcrystallites having their symmetry axis at  $54.7^\circ$  relative to the applied magnetic field. However, since samples with high cholate concentration in both the lamellar and the "hexagonal" phases exhibit narrow  $^1\text{H}$  NMR peaks, the effective relaxation time  $T_2$  is sufficiently long to make

NMR diffusion measurements feasible for these samples. For two such samples ("hexagonal" and lamellar) we have thus determined the diffusion coefficient. The results obtained are summarized in Table III, together with some previously reported data for the cubic phase. It can be seen that the diffusion rate is about a factor two slower in the "hexagonal" phase than in the lamellar phase.

#### E. X-ray diffraction

The x-ray diffraction examination was restricted to samples from the hexagonal and lamellar phase regions. Both polarizing microscopy and x-ray supported the conventional structural interpretation of the lamellar phase.

A number of samples in the hexagonal phase were investigated by low angle x-ray diffraction methods.

The ratios between lecithin and sodium cholate were the same as for the series studied by Small et al.<sup>5</sup>, viz. 70:30 and 60:40. Some of our samples contained D<sub>2</sub>O as they were also used for NMR studies (in calculation the structural parameters from the basic x-ray findings the compositions were converted on molecular basis to values for H<sub>2</sub>O containing samples). The appearance of the samples in the polarizing microscope was that of "middle phase" specimens, thus indicative of a two-dimensional hexagonal rod-structure. The x-ray diffraction findings, however, very seldom gave unambiguous support for this interpretation due to the paucity of reflections, and those obtained were furthermore weak and blurred. On the other hand the results did not contradict a hexagonal interpretation. The obtained values for the hexagonal lattice parameters agreed rather closely with those reported by Small et al.<sup>5</sup>. One general trend, however, was that our values were somewhat larger, but it is uncertain if the difference was significant.

The diffraction pattern has thus a less clear interpretation than for a typical hexagonal phase in a soap-water system. In very few cases was the typical hexagonal sequence of reflexions  $1:1/\sqrt{3}:1/\sqrt{4}$  obtained. In addition the diffraction pattern contained often additional reflexions that did not fit the hexagonal symmetry. A typical recording is shown in Fig. 7, where for comparison also films from a soap system are given. As our samples had the same bile salt to lecithin ratio as the series studied by Small et al., and were located well inside to the phase region, and special care was taken of equilibrating the samples, it seems unlikely that the additional reflexes are due to contamination of an other phase. We have not been able to analyze the additional reflexes in detail but they seem to indicate either that there is not perfect hexagonal symmetry or that additional symmetries are present. The hexagonal x-ray data are summarized in Table IV.

## DISCUSSION

### Comparison of the molecular order between the lamellar and the "hexagonal" phases

Both the proton and the deuteron NMR data strongly indicate that the molecular order is very similar in the lamellar and the "hexagonal" phases of the lecithin-sodium cholate system. This conclusion is based on the similarities between the deuteron quadrupole splittings of heavy water and of deuterated alkyl chains (Figs. 3, 4; Tables I, II) for lamellar and "hexagonal" phases. Furthermore, the proton bandwidth is approximately equal for the two phases (Figs. 2b and c). This behaviour is qualitatively different from that in ordinary soap-water systems, where generally the order parameter differs by a factor of two between the lamellar and hexagonal phases<sup>6,37</sup>. Now, the structure models proposed by Small et al.<sup>4</sup> (Fig. 1) in fact predict that there should be a similarity in the molecular packing in the



two phases, since the "hexagonal" phase is built up of coin-like lamellae stacked on each other. This can be done without changing the average orientation of the molecules in the two phases. The qualitative picture that can be obtained from the proton and the deuteron NMR data are thus in favour of Small's models.

#### The dependence of molecular order on the composition

Deuteron quadrupole splittings are particularly useful for investigations of the molecular order in lipid systems<sup>30</sup>. They can be used for a quantitative description of the dependence of the molecular order on the variation of the cholate concentration in the system presently studied. Generally it is found that the molecular order is very sensitive to the ratio between lecithin and cholate. From Table I it can be inferred that the order parameter for the C<sub>8</sub>-position of the alkyl chain of dimyristoyl lecithin is a factor three smaller for a molar ratio of 3:2 between lecithin and cholate than it is for a bilayer containing lecithin only. From Fig. 3 it can be seen that for the terminal methyl group of dilauroyl lecithin a corresponding change in the cholate concentration leads to an increase in the order parameter by a factor of approximately three. A similar trend is shown also by the water molecules (Table II), and from an analysis of the proton spectra changes of the same order of magnitude in the order parameter are obtained. It is interesting to note that cholesterol<sup>30</sup> contrary to cholate increases the ordering at all positions of the carbon chains of lecithins. This can probably be ascribed to a decrease in the flexible motion of the alkyl chains caused by the interaction with the rigid steroid skeleton of the cholesterol molecule. It is thus expected that also the cholate skeleton should have a similar influence on the lecithin alkyl chains provided that the molecule is incorporated in the same way as

cholesterol in the bilayers. Therefore the experimental data obtained are not compatible with the structural models shown in Fig. 1. A better model of the incorporation of the cholate molecules in the lamellar phase consistent with the order parameters is drawn in Fig. 8a. Here the cholate molecules are solubilized more or less randomly in the bilayer. The hydrophilic nature of one side of the cholate molecule is accounted for either by placing the molecule on the surface or by solubilizing it as a dimer (or larger aggregates) in the lamellae.

From the general theory of surfactant systems<sup>7,38</sup> it also follows that a lamellar structure will be broken down by increasing the ratio between the area of the polar groups and the volume of the alkyl chain.

If a cholate ion lies flat on the lamellar surface there will be a force introducing a non-planarity (see Fig. 8). Thus both the disordering of the alkyl chains and the non-planarity will tend to decrease the order parameter. Also in the "hexagonal" phase a considerable perturbation of the packing of the lecithin alkyl chains must occur. The model of Fig. 1 can be made consistent with this observation if the mixed micelles have a shape more like a lense than of an ideal cylindrical segment. This model is, however, not compatible with the NMR diffusion data, since no restricted diffusion was observed.

#### Models of the molecular structure of the different liquid crystalline phases

The large decrease in the molecular order occurring on addition of cholate seems to indicate that at least some of the cholate molecules are in some way located at the aggregate surface of both the

lamellar and "hexagonal" aggregates. Thus for the latter the cholate molecules cannot cover the hydrophobic surface of the lamellar coin-like lecithin aggregates but they are probably solubilized in a similar way as in the lamellae. Whether a curvature of the bilayers is also induced remains an open question. However, the unusually small water quadrupole splitting at high cholate concentrations supports the view of a curvature. Here the water molecules can diffuse over the bent lamellae leading to a decrease in the splitting. Furthermore, the deuteron methylene splittings for the lamellar and "hexagonal" phases are very similar. Although some of our NMR data are compatible with Small's model in Fig. 1 for the "hexagonal" phase, with slight modifications, the results of the diffusion experiments are not since they indicate amphiphile diffusion over a continuous aggregate. A new model must be constructed. The x-ray diffraction data indicate (see Fig. 7) the presence of additional symmetries in the "hexagonal" phase. This extra symmetry can be explained by either the periodic stacking of mixed micelles or by long more or less flat rods. Together with the NMR diffusion data the latter possibility is to be preferred. In Fig. 8 we have constructed a tentative model of both the lamellar and the "hexagonal" phases, showing possible locations of the cholate molecules. These can be solubilized as dimers, trimers etc or as monomers at the aggregate surfaces.

For the cubic phase the diffusion data of ref. 14 also shows the presence of continuous amphiphile aggregates. Different possible cubic structures were recently discussed by Scriven<sup>39</sup> and it seems that Neovius' cubic structure (Fig. 9) have the geometrical characteristics that are compatible with the molecular arrangement proposed in Fig. 8.



ACKNOWLEDGEMENT

This work was supported by the Swedish Natural Science Research Council.

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Table I. The deuteron quadrupole splitting for the  $C_8$ -position of dimyristoyllecithin for lamellar and "hexagonal" phases. Temperature 28°C.

| $\Delta$ , kHz | Phase       | Molar ratio<br>lecithin/choleate |
|----------------|-------------|----------------------------------|
| 9              | "hexagonal" | 1.6                              |
| 9              | lamellar    | 1.6                              |
| 27             | lamellar    | $\infty$                         |

Table II. Water quadrupole splittings obtained for the lamellar and "hexagonal" phase of the system egg yolk lecithin-sodium cholate-heavy water. Temperature 28°C.

| Sample composition |           |                  | Phase       | $\Delta$ , Hz |
|--------------------|-----------|------------------|-------------|---------------|
| EYL                | NaCholate | D <sub>2</sub> O |             |               |
| 31.0               | 13.0      | 56.1             | "hexagonal" | 110           |
| 37.8               | 15.8      | 46.4             | "           | 160           |
| 42.8               | 18.1      | 39.1             | "           | 200           |
| 40.5               | 22.0      | 37.5             | "           | 220           |
| 70.6               | 3.5       | 25.9             | lamellar    | 600           |
| 46.4               | 6.3       | 47.3             | "           | 240           |
| 60.3               | 17.3      | 22.4             | "           | 210           |

Table III. Diffusion coefficients determined for lecithin in different liquid crystalline phases in the egg yolk lecithin-sodium cholate-water system

| Sample composition (% w/w) |      |      | Phase                        | Temperature | $D \text{ m}^2/\text{s} \cdot 10^{11}$ |
|----------------------------|------|------|------------------------------|-------------|----------------------------------------|
| lecithin:cholate:water     |      |      |                              |             |                                        |
| 50.0                       | 26.0 | 24.0 | cubic                        | 24          | 0.1                                    |
| 55.4                       | 23.4 | 21.2 | lamellar<br>(oriented)       | 23          | 0.9                                    |
| 55.4                       | 23.4 | 21.2 | lamellar<br>(oriented)       | 26          | 1.1                                    |
|                            |      |      | "                            | 39          | 1.7                                    |
| 66.6                       | 9.2  | 24.2 | lamellar<br>(oriented)       | 23          | 0.8                                    |
| 57.6                       | 21.3 | 21.1 | lamellar<br>(nonoriented)    | 26          | 1.2                                    |
|                            |      |      | "                            | 38          | 1.6                                    |
|                            |      |      | "                            | 47          | 1.7                                    |
| 34.0                       | 24.6 | 41.4 | "hexagonal"<br>(nonoriented) | 26          | 0.5                                    |
|                            |      |      | "                            | 38          | 0.9                                    |
|                            |      |      | "                            | 47          | 1.1                                    |

Table IV. X-ray findings for the hexagonal mesophase.

| EYL  | Composition (%) |                  | The hexagonal<br>lattice parameter<br>dp (Å) | The diameter<br>of the rod-<br>aggregates<br>dc (Å) | The area per<br>molecule at<br>the polar/non-<br>polar interface<br>S (Å) <sup>2</sup> |
|------|-----------------|------------------|----------------------------------------------|-----------------------------------------------------|----------------------------------------------------------------------------------------|
|      | NaCholate       | H <sub>2</sub> O |                                              |                                                     |                                                                                        |
| 34.6 | 14.8            | 50.6             | 60.4                                         | 44.6                                                | 94.8                                                                                   |
| 39.1 | 16.8            | 44.1             | 58.1                                         | 45.6                                                | 92.7                                                                                   |
| 44.6 | 19.1            | 36.3             | 56.4                                         | 47.3                                                | 89.4                                                                                   |
| 35.9 | 23.9            | 40.2             | 48.7                                         | 39.6                                                | 99.9                                                                                   |
| 40.3 | 26.9            | 32.8             | 46.8                                         | 40.3                                                | 98.2                                                                                   |

The hexagonal rod-structure is assumed to be the "classical" one, viz. homogeneous amphiphilic rods arranged hexagonally in an aqueous environment. All molecules are assumed to be anchored at the interfaces between the polar and non-polar regions. The molecular weight of the lecithin is taken as 800 and the density of both the polar and non-polar regions of the structure has been assumed to be unity.



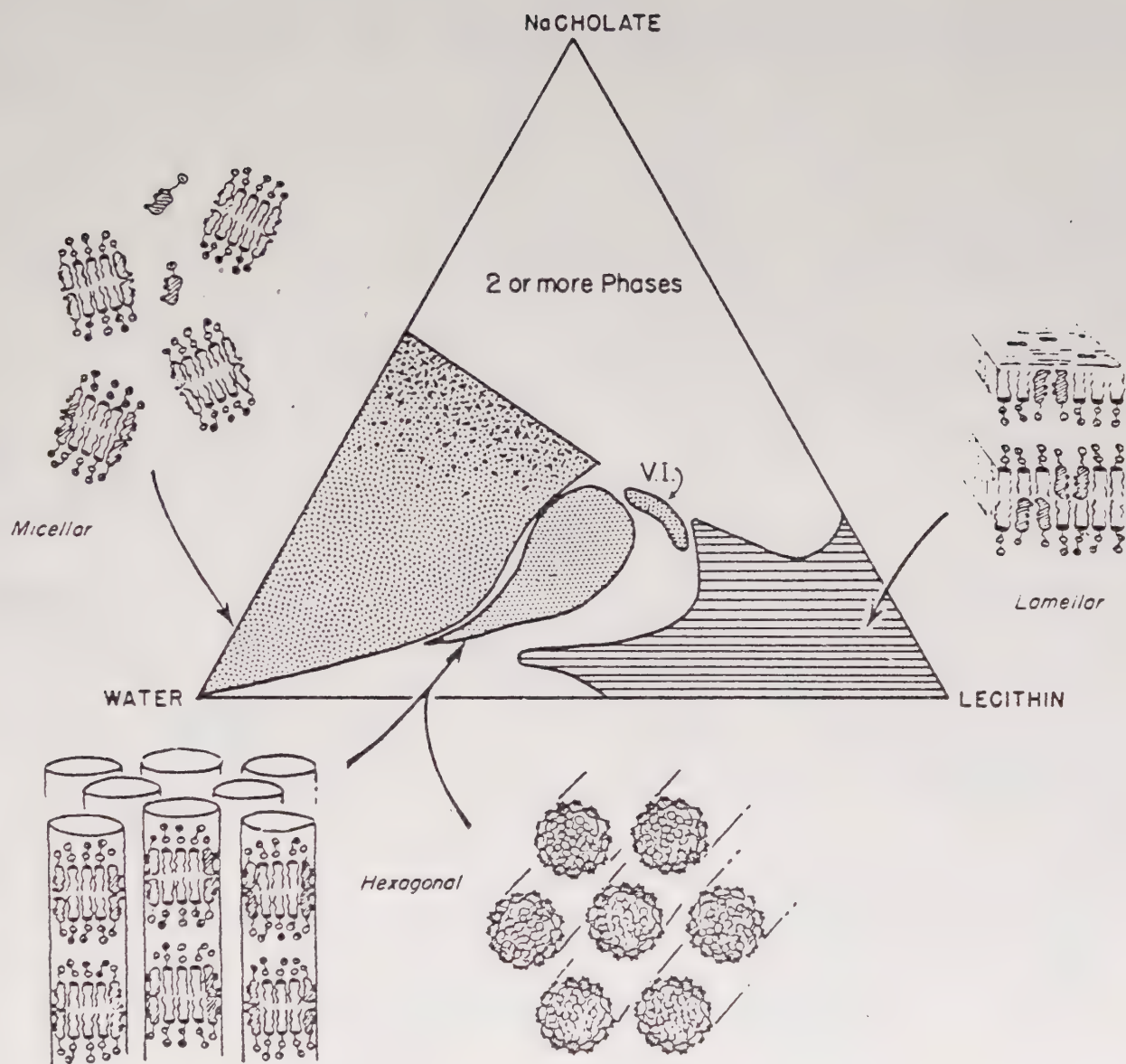


Figure 1 The phase diagram for the ternary system egg yolk lecithin-sodium cholate-water at 22°C. The structures for the different phases suggested by Small<sup>1</sup> are indicated. (Reproduced from ref. 1).

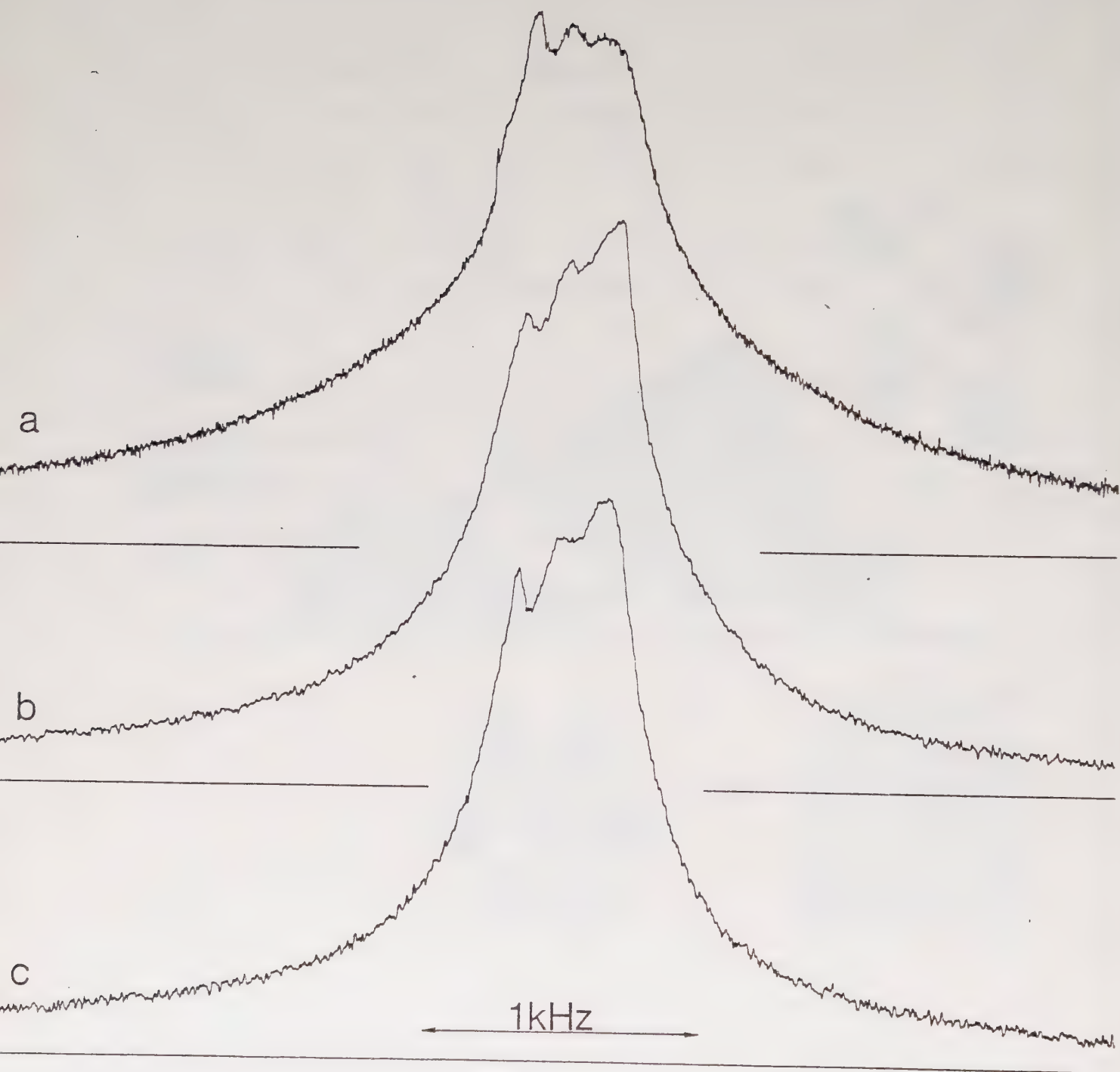


Figure 2 100 MHz  $^1\text{H}$  NMR spectra for a) and b) lamellar phase and c) "hexagonal" phase at  $28^\circ\text{C}$ . The composition egg yolk lecithin: sodium cholate:  $^2\text{H}_2\text{O}$  (% w/w) is a) 66.6:9.2:24.2, b) 55.4:23.4:21.2 and c) 37.8:15.8:46.4.

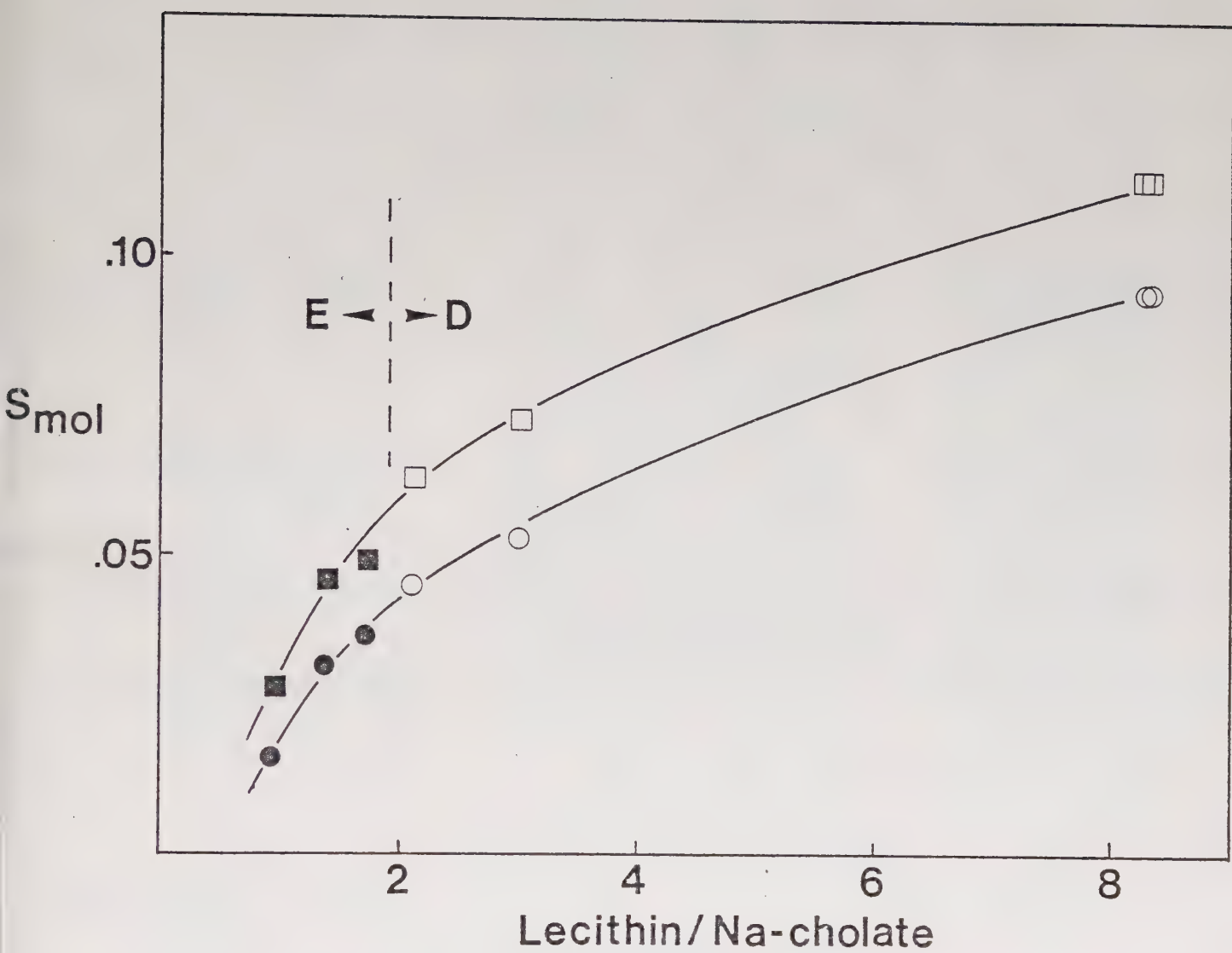


Figure 3 The deuterium order parameter  $S_{mol}$  of  $\omega$ - $d_3$ -dilauroyl lecithin as a function of the molar ratio between lecithin and sodium cholate. Temperature: 28°C.



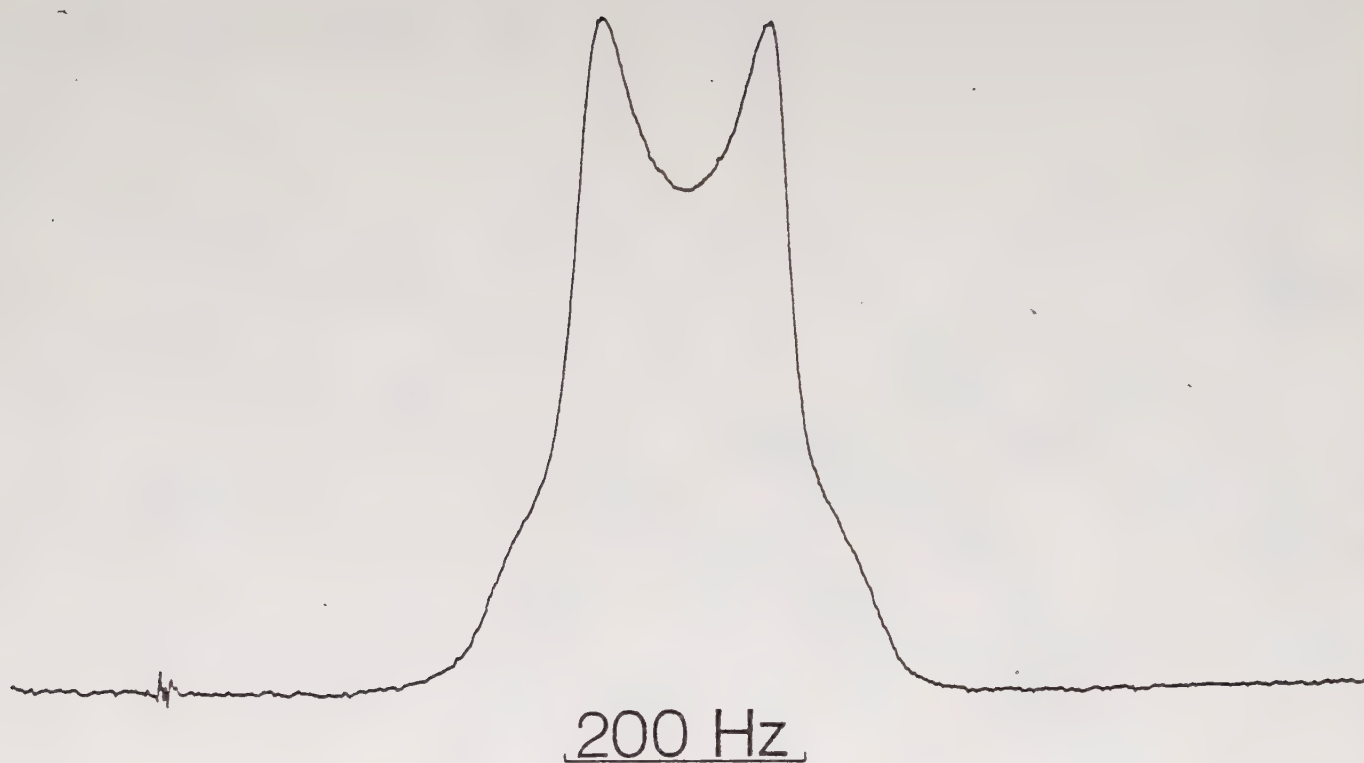


Figure 4     15.4 MHz  $^2\text{H}$  spectrum of  $^2\text{H}_2\text{O}$  for a sample from the "hexagonal" phase with the composition (% w/w) egg yolk lecithin: sodium cholate:  $^2\text{H}_2\text{O}$  42.8:18.1:39.1 at 28°C.

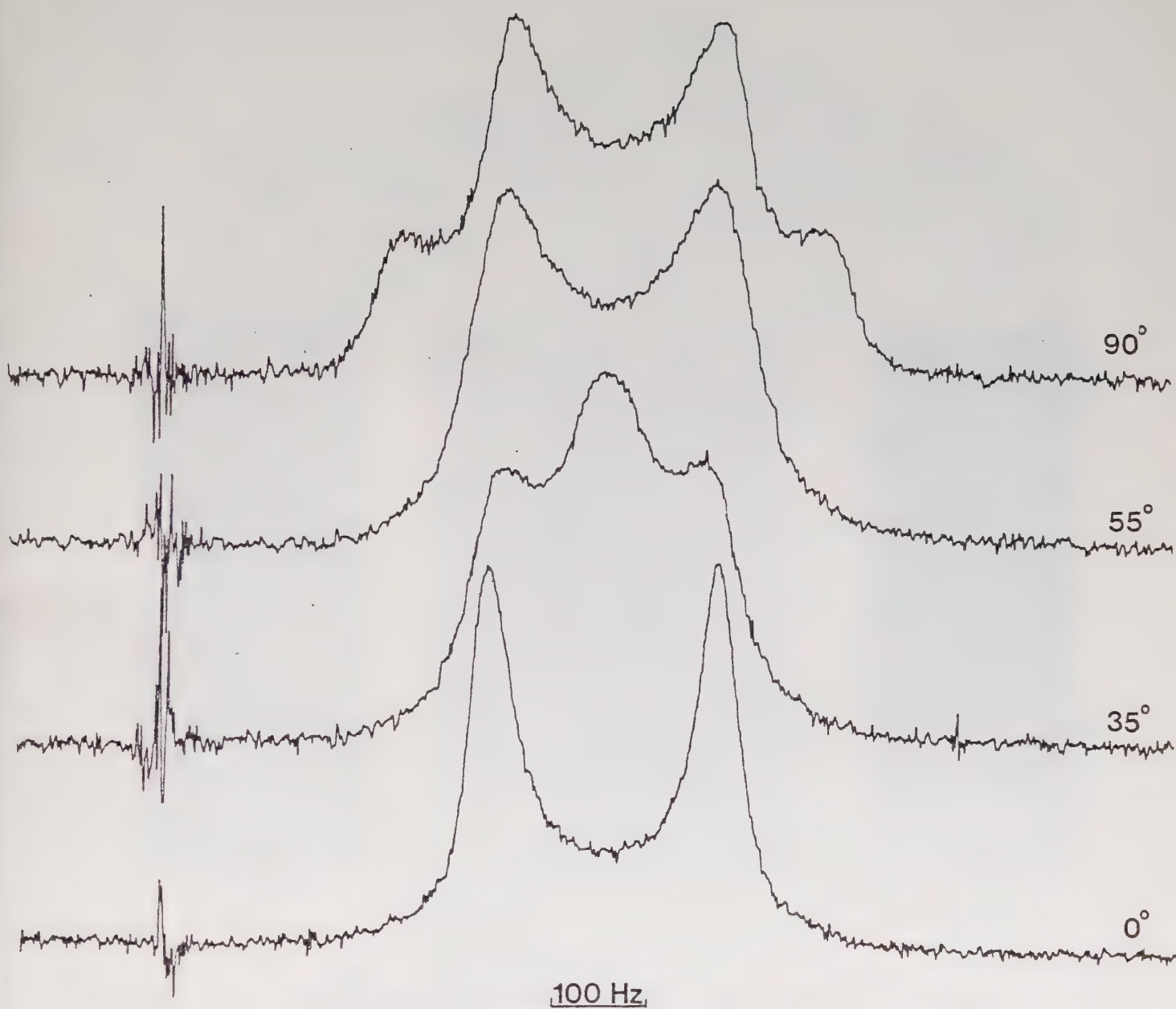
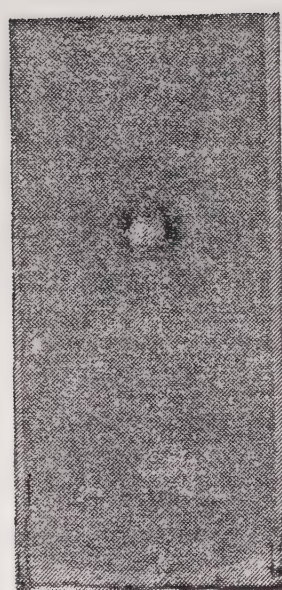
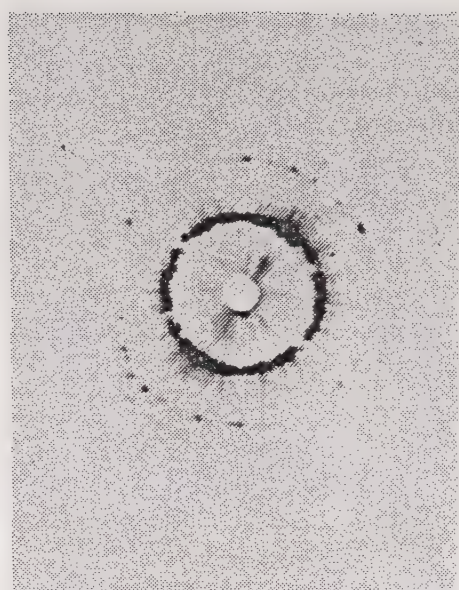


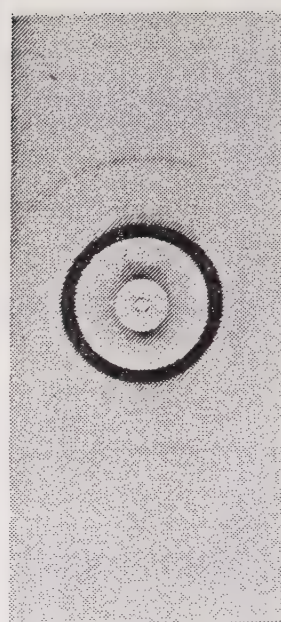
Figure 6 15.4 MHz  $^2\text{H}$  water spectra of an oriented "hexagonal" phase sample. The angles given are between the normal to the glass plates and the magnetic field. Temperature: 28°C.



A



B1

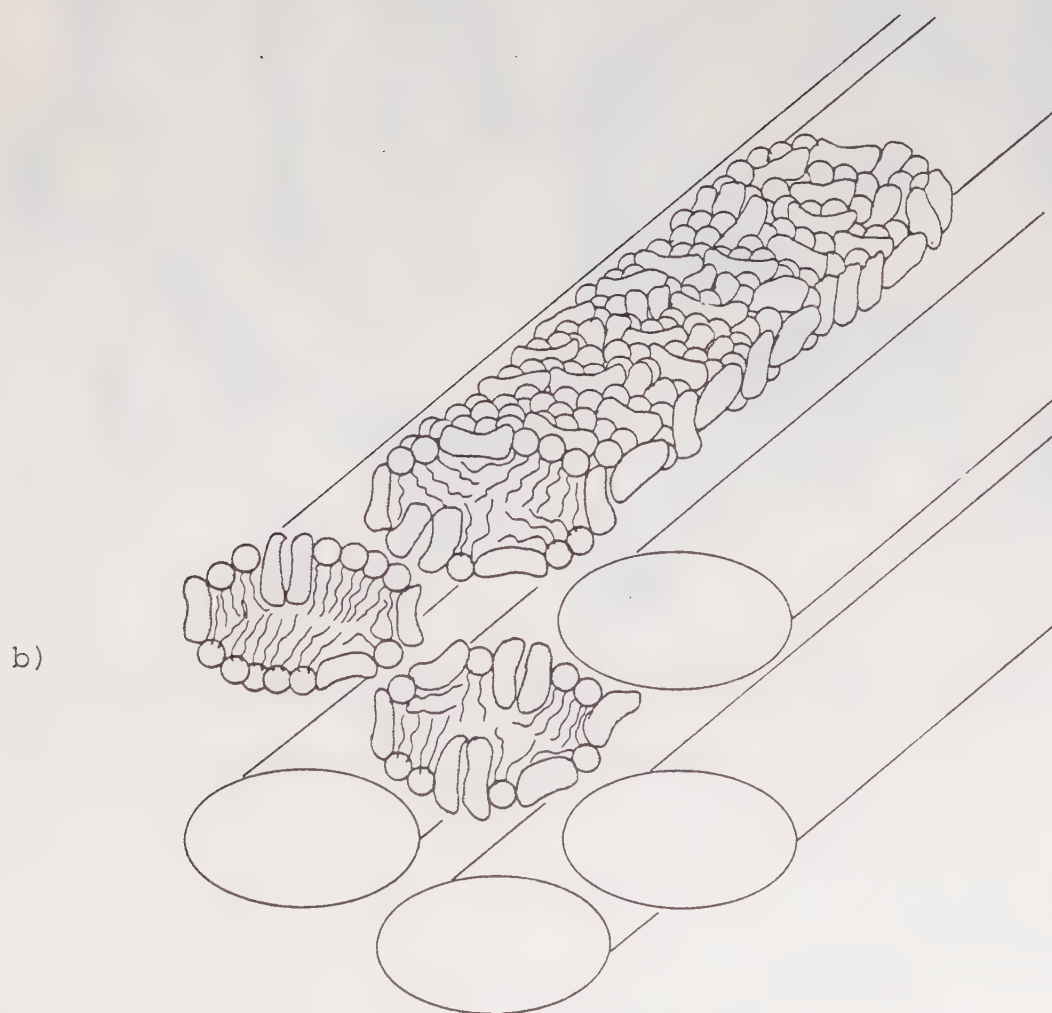
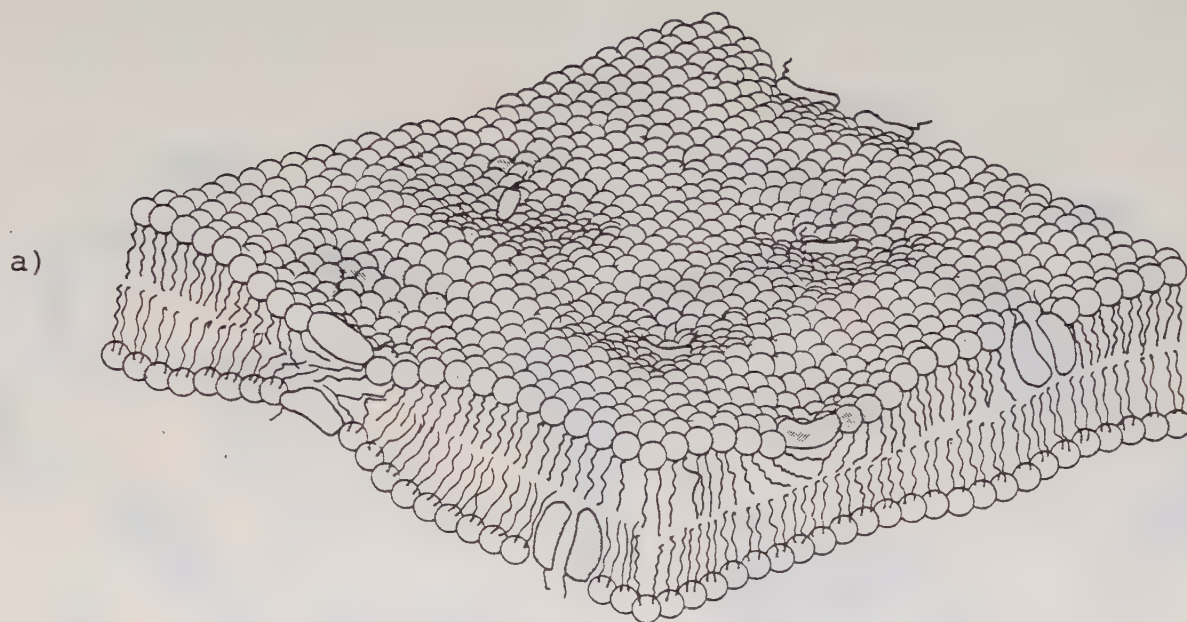


B2

Figure 7 A) Typical low-angle x-ray diffraction patterns from the "hexagonal" phase. Sample composition egg yolk lecithin: sodium cholate : water (% w/w) 40.3 : 26.9 : 32.8.

B) As comparison are given x-ray diffraction pictures for hexagonal 1) and 2) lamellar phases in the sodium octanoate-decanol-water system (23).

Figure 8      Suggested structures for a) the lamellar phase and  
b) for the hexagonal phase of the system lecithin-  
-sodiumcholate-water.





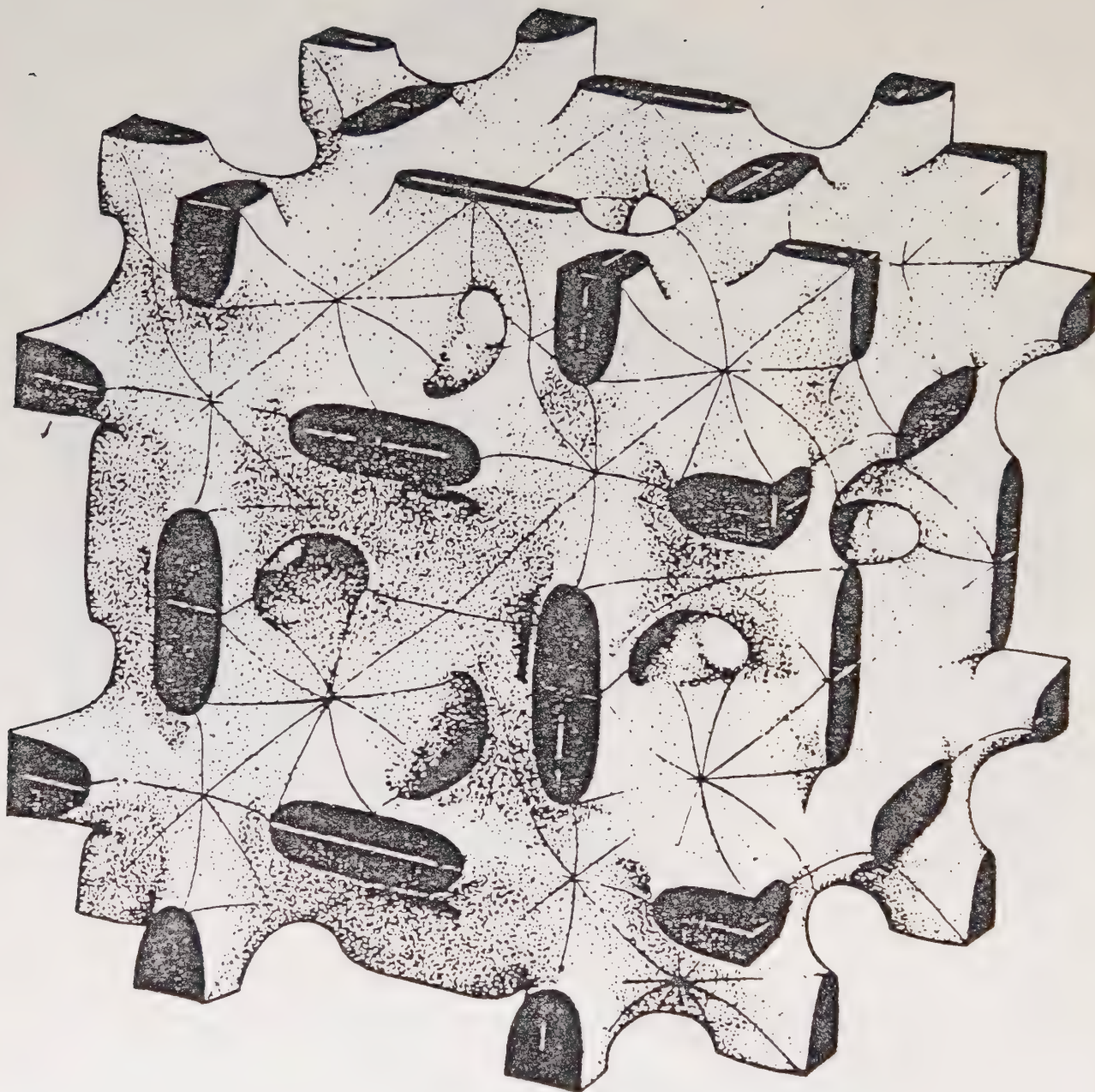


Figure 9      Neovius' cubic structure suggested for the cubic phase in the system lecithin-sodium cholate-water. (Reproduced from ref. 39).





VISCOELASTICITY IN SURFACTANT SOLUTIONS. CHARACTERIZATION  
OF THE MICELLAR AGGREGATES AND THE FORMATION OF PERIODIC  
COLLOIDAL STRUCTURES

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## Abstract

The properties of viscoelastic dilute aqueous solutions containing the hexadecyltrimethylammonium cation ( $\text{CTA}^+$ ) is investigated, monitoring the proton magnetic resonance spectrum and the linear dichroism induced in a shear gradient. It is found that the viscoelastic behaviour of the dilute solutions correlates, seemingly in a one to one way, with the formation of large rod-shaped micellar aggregates. This correlation is manifest both with respect to changes in composition and in temperature. It is suggested that the behaviour of the solutions is caused by the presence of a periodic colloidal structure formed due to the repulsive force between the aggregates. The formation of such periodic structures has recently been proposed on theoretical grounds for dilute polyelectrolyte solutions.

## Introduction

Aqueous solutions of some amphiphilic molecules show a striking viscoelastic behaviour<sup>3</sup>. The viscoelasticity can for example be seen by simply swirling the solution and visually observing the recoil of air bubbles trapped in the solution after the swirling is stopped. The viscoelasticity is manifested in a number of other properties as, for example, a non-Newtonian viscous behaviour<sup>1a,1b</sup> and a flow induced optical anisotropy as illustrated in Figure 1<sup>1b</sup>.

Although viscoelasticity<sup>2</sup> has been observed for a number of aqueous solutions containing ionic amphiphiles (see ref. 3 and references cited therein) the property is of a rather rare occurrence. Furthermore the chemical difference between solutions which show viscoelasticity and those which do not is sometimes small<sup>3</sup>. Usually the viscoelastic behaviour is observed when a third component is added to a rather dilute (< 1 % w/w) aqueous solution of an ionic amphiphile. Recently one of us<sup>3</sup> found that also some two component systems containing the hexadecyltrimethylammonium cation (CTA<sup>+</sup>) and some organic anions, such as the salicylate ion, show a pronounced viscoelasticity. In Figure 2 the viscoelastic region of the CTA-salicylate system is shown. In trying to elucidate the mechanism behind the viscoelastic behaviour it is a considerable simplification to study a two component system rather than a three component one. The experimental studies in the present work is therefore mainly concentrated on the CTA-salicylate system.

In order to obtain an insight into the mechanism behind the viscoelastic behaviour the size and shape of the micellar aggregates present in the solutions are discussed at first. Then the mechanism behind the chemical specificity of the phenomenon and possible mechanisms for the viscoelastic behaviour are treated. Finally

some properties of periodic colloidal structures are described.

### Size and shape of the amphiphilic aggregates in the viscoelastic solutions

A first step in an elucidation of the mechanism behind the viscoelastic behaviour is to characterize the amphiphilic aggregates present in the solution with respect to size and shape. The proton magnetic resonance spectrum of the protons in the alkyl chain of the amphiphile is sensitive to the presence of large aggregates and a detailed analysis of the proton NMR spectrum provides information about the reorientational correlation time of the aggregates<sup>4,5</sup>. Figure 3 shows the  $^1\text{H}$  NMR spectra of 12 mM solutions of (a) CTA-salicylate and (c) CTA-p-hydroxybenzoate. Only the former solution is viscoelastic. For the CTA-salicylate system the broad spectral lines indicate a long correlation time and consequently the presence of large aggregates. Using the same procedure as in ref. 5, and taking into consideration that the exchange of amphiphile molecules between aggregates of different size is slow on the NMR timescale, the spectrum 3a is simulated as shown in Figure 3b to obtain the reorientational correlation time  $\tau_r$ . This procedure was repeated for several temperatures and the resulting correlation times are represented in Figure 4 as a function of the temperature. By assuming that the aggregates are stiff noninteracting rods one can relate  $\tau_r$  to the length of the rod<sup>6</sup>. The calculated lengths are also plotted in Fig. 4. It is seen that the loss in viscoelastic behaviour around 50°C (cf. Figure 2) is accompanied by a drastic decrease in aggregate size.

In the analysis of the proton NMR spectrum it was tacitly assumed that the micellar aggregates were rodshaped. There are several observations that support this assumption. At higher



concentrations of the amphiphile, but within the optically isotropic phase, the  $^1\text{H}$  NMR spectra are the same as those observed for hexagonal phases containing the  $\text{CTA}^+$  ion as the amphiphile (cf. ref. 5, Figure 1 d). Furthermore in the CTA-salicylate system a hexagonal phase is formed above a concentration of 30 % showing that the amphiphile preferentially forms rod-shaped aggregates<sup>7,8</sup>. The anisometric nature of the aggregates present in the CTA-salicylate solutions was also demonstrated by studying the linear dichroism (LD) in the UV spectrum of the salicylate anion induced by a shear gradient<sup>9,10</sup>, which produces a partial orientation of anisometric aggregates<sup>9,11</sup>. Figure 5 shows the temperature variation of the linear dichroism for three different amphiphile concentrations. A comparison with Figure 2 reveals that the occurrence of an LD signal correlates very well with the occurrence of viscoelastic behaviour. It is also seen that the LD signal increases with increasing amphiphile concentration.

From the NMR and LD measurements we conclude that the viscoelastic behaviour of these dilute amphiphile solutions correlates, seemingly in a one to one fashion, with the presence of large rodshaped micellar aggregates. This qualitative relation has been suggested previously by several authors<sup>12-16</sup>. Furthermore the values of  $\tau_r$  in Fig. 4 show that the aggregates are not exceedingly large and that the reorientation of the aggregates, in an unperturbed system, is much faster than the relaxation of the elastic motion. It also follows that the existence of a three-dimensional network of thin rods is not compatible with the experimental observations. The measured  $\tau_r$  seems too short for such a system and  $\tau_r$  has also the wrong concentration dependence. (It increases with increasing amphiphile concentration.) In a network structure the distance over which the system is isotropic decreases with increasing concentration. In the LD experiment one would expect the more dilute solutions with a looser network to be more easily oriented than those of a more concentrated solution. As seen from Figure 5 this is not the case.

## The chemical specificity of the viscoelastic behaviour

The viscoelastic behaviour shows a considerable chemical specificity both with respect to counterions<sup>3</sup> and additives<sup>17,18</sup>. From such observations one would suspect that specific chemical interactions are directly responsible for the mechanism behind the viscoelasticity. However the NMR spectra in Figure 3 of a viscoelastic and a non viscoelastic solution indicate that the difference between the salicylate and p-hydroxybenzoate counterions is that the former can induce the formation of large aggregates in the CTA<sup>+</sup> system while the p-hydroxybenzoate cannot. A similar observation was made by Larsen et al.<sup>17</sup>. This role of the counterions and the additives is also in accordance with the conclusions in the previous section.

In recognizing the molecular features of additives and counterions that are essential for the formation of large aggregates a detailed analysis of the aggregation of amphiphiles to micelles is helpful. A quantitative model for how micelles grow in size with increasing surfactant concentration was recently presented by Israelachvili et al.<sup>19</sup>. In their model the growth in size of micelles is determined by the asymptotic behaviour of the chemical potential of the aggregates, which for an aggregate that grows in one dimension can be written<sup>19</sup>

$$\mu_N^\theta = \mu_\infty^\theta + \frac{\alpha RT}{N} \quad (1)$$

Here  $\mu_N^\theta$  is the chemical potential per monomer in an aggregate of N monomers and  $\alpha$  is a (temperature dependent) parameter characteristic for the particular system. The inverse dependence on N is obtained if the change in chemical potential with aggregate size is determined by the unfavourable packing of the monomers at the ends of the aggregate. When  $\alpha$  is nega-

tive or positive and small the micelles stay essentially spherical, while large positive values of  $\alpha$  implies a rapid increase in micelle size and concomittantly a large polydispersity in the size distribution<sup>19</sup>. An additional point in the analysis is that as long as the limiting value  $\mu_{\infty}$  is lower for a cylindrical aggregate than for a lamellar one, the aggregates will grow continuously in size and a transition to a liquid crystalline phase will not occur until interaggregate interactions become important<sup>8,19</sup>.

For a given amphiphile, such as the CTA<sup>+</sup> ion, the factors that influence  $\alpha$  in eq. (1) is the counterion and the presence of additives. Tanford<sup>20,21,22</sup> has pointed out that a useful characterization of an amphiphilic molecule is obtained by specifying the length  $l_0$  of the hydrocarbon chain, the cross-sectional area  $a_0$  of the polar head and the hydrocarbon volume  $v_0$ . Since the area volume ratio decreases in the sequence spherical, cylindrical and planar aggregates for a given value of  $l_0$  a large  $a_0/v_0$  ratio favours a spherical while a small ratio favours a planar aggregate with the cylindrical one falling in between.

The strong ion binding to ionic micelles is one of the important factors in determining the energetics of micellization<sup>7</sup>. For a rod-shaped micelle the surface charge density is larger than for a corresponding spherical one and the counterion binding is consequently larger<sup>7,23</sup>. If, in addition to the electrostatic effects which are equal for all counterions, there is an attractive chemical contribution to the ion binding, such counterions will increase  $\alpha$  in eq. (1). Is the counterion in itself amphiphilic such a chemical contribution obviously exists. This is the case for the viscoelastic mixtures of cationic and anionic amphiphiles studied by Tiddy and coworkers<sup>15,16</sup>. This also occurs for the viscoelastic fluorocarbon soap systems studied by Hoffmann et al.<sup>24</sup>. There is also the possibility that the aromatic part of



salicylate anion is solubilized by the micelle while the polar part is at the hydrophilic surface. For the p-hydroxybenzoate anion a solubilization of the aromatic ring forces the hydroxyl group into an unfavourable apolar surrounding.

The role of additives is somewhat more complex to analyze. An aliphatic compound like cyclohexane is solubilized in the interior of the micelle<sup>23,25</sup> and it will increase the hydrocarbon volume but also the effective length of the amphiphile. The latter effect is the most important one and the micelles stay small<sup>23,25,26</sup>. An aliphatic alcohol, such as hexanol, is solubilized with the polar group at the aggregate surface. This leads to an increase in  $\alpha$  in eq. (1) but the occurrence of very large micelles is in this case masked by the appearance of a liquid crystalline phase<sup>8</sup>. Aromatic compounds are for lower additive/amphiphile ratios solubilized close to the polar surface of the micelles<sup>7,25,28,29</sup> while at higher ratios positions inside the micelles are also occupied.<sup>29</sup> Thus initially rod-shaped aggregates are favoured and the micelles are large but further addition of the aromatic additive (when possible) seems to reduce the micelle size again<sup>26,29,30</sup>. This effect manifests itself in a spectacular way when 1-methylnaphtalene is added to a CTA<sup>-</sup> salicylate solution. The system then loses its viscoelastic properties in spite of the fact that the same additive induces viscoelasticity in a CTA-Br solution.

#### Possible mechanisms for the viscoelastic behaviour

The knowledge about the molecular arrangements in the viscoelastic solutions developed in the previous sections provides a basis for a discussion of possible mechanisms behind the viscoelasticity. Since the viscoelastic solutions considered here contain micellar aggregates in an optically isotropic solution the suggestion that the viscoelasticity is due to the formation of a (micro)emulsion<sup>13</sup> can be ruled



out. As stated in section 2 the idea that the rod-shaped micelles aggregate to form a closed network<sup>12</sup> is not compatible with the experimental observations. That the micellar aggregates can influence the water structure on a long range scale<sup>3</sup> has very little support in experimental studies of water amphiphile systems<sup>7,31,32</sup>.

That the rod-shaped micelles are oriented during flow and in this way store elastic energy<sup>15,16</sup> is a mechanism very similar to the one known to cause viscoelasticity in polymer solutions<sup>33,36</sup>. For a rigid rod (length,  $L$ , diameter  $d$ ) the relaxation time  $\tau$  characterizing the elastic mode is<sup>37</sup>

$$\tau \equiv \frac{\pi \eta_s L^3}{18 kT} \ln(2 L/d) \quad (2)$$

where  $\eta_s$  is the viscosity of the solvent and  $k$  is Boltzmann's constant. For the values of  $L$  given in Fig. 4 the calculated  $\tau$  is of the order of  $10^{-6}$  s. (It should be of the same order of magnitude as reorientational correlation time measured in the NMR experiment). This mechanism will certainly be operating in solutions of rod-shaped micelles, but the estimated relaxation time is far too small to account for the visually observed viscoelasticity, where the relaxation times must be of the order of seconds or more. As the examples with polymer solutions show, the introduction of flexibility into the rods does not change the qualitative nature of the viscoelasticity.

A typical property of the viscoelasticity of the surfactant solutions is the long range spacial correlations present in the system as exemplified in Fig. 1. It is also clear that the solutions have a memory that can last for seconds. This implies to us that a long-range order is present in the solutions and since the elastic behaviour is manifest even for very small perturbations this long range order is present even when the solutions are at rest. Thus we suggest that in the viscoelastic solutions the micellar aggregates form a periodic optically isotropic structure that extends over macroscopic distances<sup>38,39</sup>.

## Periodic structures in colloidal systems

For a particle in a fluid the translational energy is  $3/2 kT$ . This is true irrespective of particle size and is thus equally valid for a water molecule and a large micell aggregate. As a consequence much weaker (on a mass basis) forces are needed to introduce a correlation between large particles than between small ones. This principle lies behind many of the odd mechanical properties of colloidal systems and periodic colloidal structures are frequently seen<sup>38,39</sup>. Particularly for charged latex spheres the existence of periodic structures has been clearly demonstrated by direct electron microscopic observation<sup>40</sup> and by light scattering experiments<sup>41,42</sup>. Particle correlation effects have also been observed by neutron scattering experiments on neutralized polymetacrylic acid<sup>43</sup>. Similarly, the first X-ray scattering experiments on concentrated micellar solutions were interpreted as due to ordered lamellar structures<sup>44-46</sup>. However, the observed diffraction peaks could be explained by the correlations between spherical micelles induced by electrostatic repulsion<sup>47</sup>.

The long range forces that are acting in colloidal systems are the electrostatic repulsion and the attractive dispersion interaction. The electrostatic repulsion is very strong at short distances but in electrolyte solutions it decays exponentially due to screening. The dispersion forces are weaker but they decay more slowly<sup>38</sup>. For highly charged systems at moderate electrolyte concentrations the evidence is that the electrostatic effects dominate<sup>42</sup>. The driving force for the formation of a periodic structure is then a minimization of the repulsive forces within the boundaries of the solvent medium. This leads to a so called restricted volume periodic structure<sup>38</sup> and for spherical particles this is of bodycentered cubic symmetry<sup>48</sup>. de Gennes et al.<sup>49</sup> recently made a theoretical analysis of the structure of

polyelectrolyte solutions in the absence of added salt. They predicted that for very dilute solutions, where the mean distance between the polyions exceeded the size of the polyion, a periodic lattice of cubic symmetry should form. At higher polyion concentrations this periodic structure is replaced by either a hexagonal structure or an isotropic solution with a smaller correlation length.

The system CTA-salicylate + water studied in the present work is viscoelastic down to concentrations of 0.19 mM (at 25°C)<sup>3</sup> which just exceeds the c.m.c. (0.15 mM at 25°C)<sup>3</sup>. To get a qualitative picture of the typical distances in the system assume that half of the amphiphilic monomer is present in the aggregates and that these contain on the average 300 monomers (axial ratio 5, length 20 nm). The aggregate concentration is then 0.3 μM and the separation between the particles is 200 nm: At this large distance the repulsion between the particles is rather weak but as the particles approach an increasingly strong repulsion will appear. A quantitative analysis of the problem is presently difficult due to an imperfect knowledge about the electrostatic interactions.

### Conclusions

The presence of a long range periodic structure seems to be the most plausible rationalization of the viscoelastic behaviour of dilute amphiphile solutions containing moderately large rod-shaped micelles. The structure has cubic symmetry but when the system is mechanically perturbed the lattice is deformed and an optical anisotropy that progresses over macroscopic distances is generated. In a shear gradient the cubic structure is deformed and when the



shear is stopped a recoil to the cubic structure occurs. The picture of a periodic structure thus accounts for many of the characteristic properties of the viscoelastic solutions in a qualitative way. However, a quantitative theory of both the conditions for the formation of a periodic structure and for the response of such a structure to mechanical perturbations has still to be developed.

### Experimental

Cetyltrimethyl ammonium bromide (CTAB) was purchased from E. Merck, Darmstadt, W. Germany and used without further purification. Cetyltrimethyl ammonium salicylate and -p-hydroxybenzoate was prepared by adding equimolar amounts of the corresponding acid (BDH, Poole, England) to a CTA-OH solution which was prepared from CTAB by ion exchange on a Dowex 21K column. 1-methyl naphthalene was purchased from BDH, Poole, England. Viscoelasticity was detected by swirling a sample of solution and visually observing the recoil of small air bubbles entrapped in the sample after swirling was stopped. Proton NMR measurements were made on solutions in  $D_2O$  using a Varian XL-100-15 NMR spectrometer operating at 100 MHz in the continuous wave mode.

Linear dichroism measurements were performed with a JASCO J-40 circular dichroism spectrometer as described previously<sup>11</sup>. The sample was subjected to a linear shear gradient in a thermostated Couette cell, consisting of two concentric cylinders separated by  $0.05 \text{ mm}$ <sup>10</sup>.



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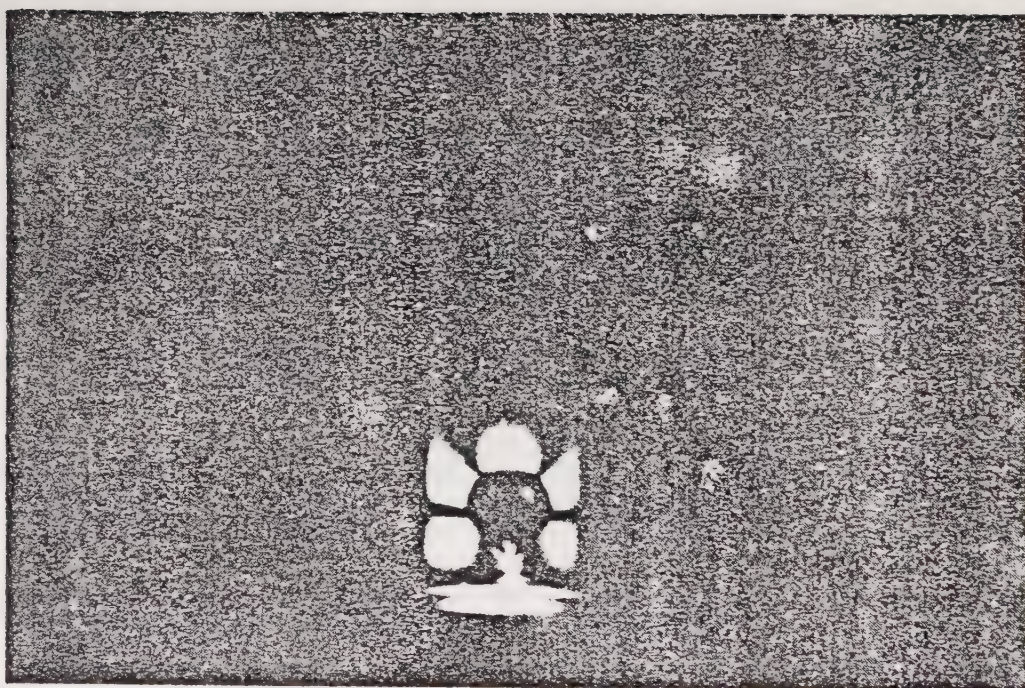


Fig. 1. Air bubble rising in a solution of 0.1 M CTABr + 0.1 M sodium salicylate<sup>16</sup>. Viewed in a polariscope.

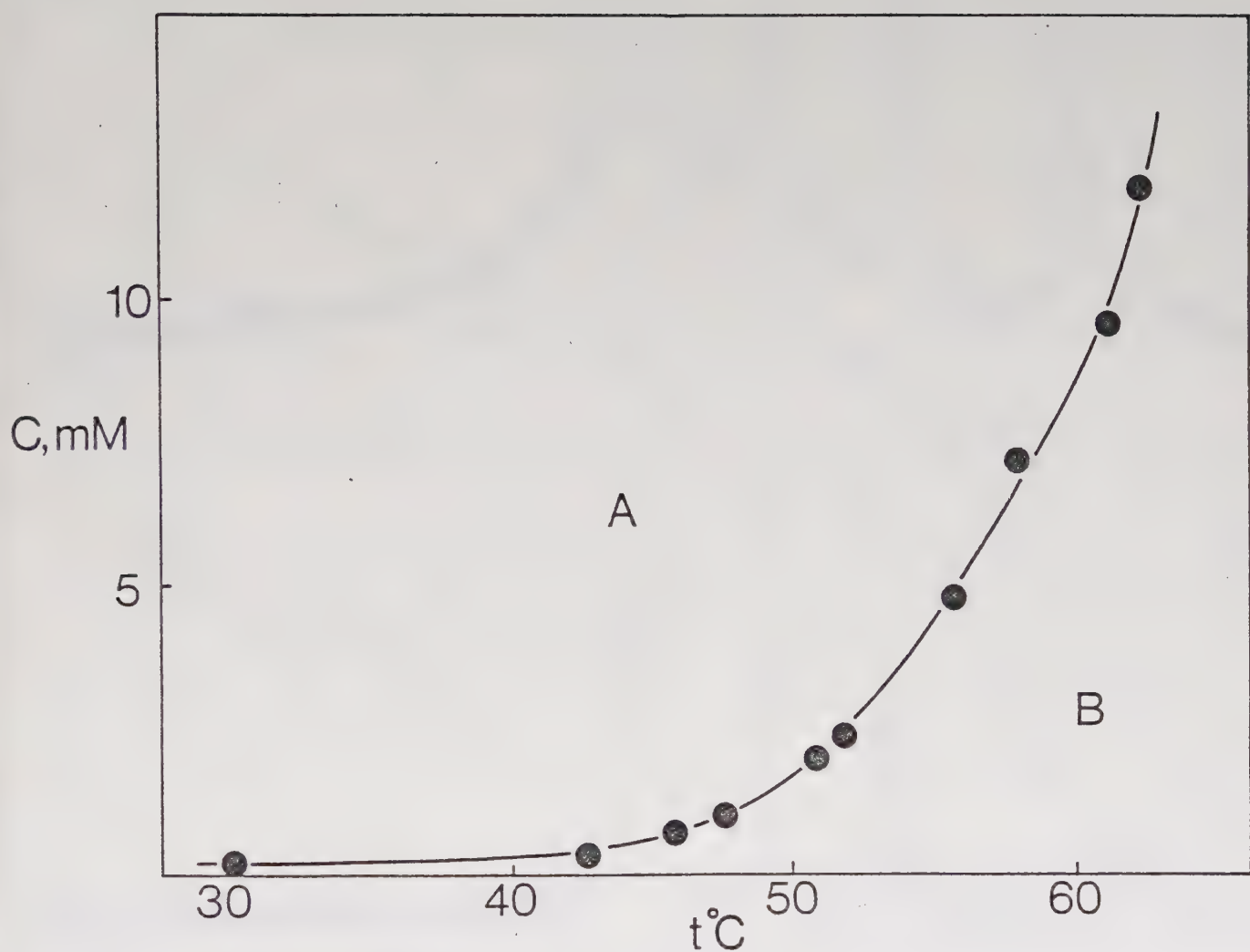


Fig. 2. The viscoelasticity of CTA-salicylate solutions as a function of concentration and temperature. Part A: viscoelastic solution. Part B: newtonian solution.

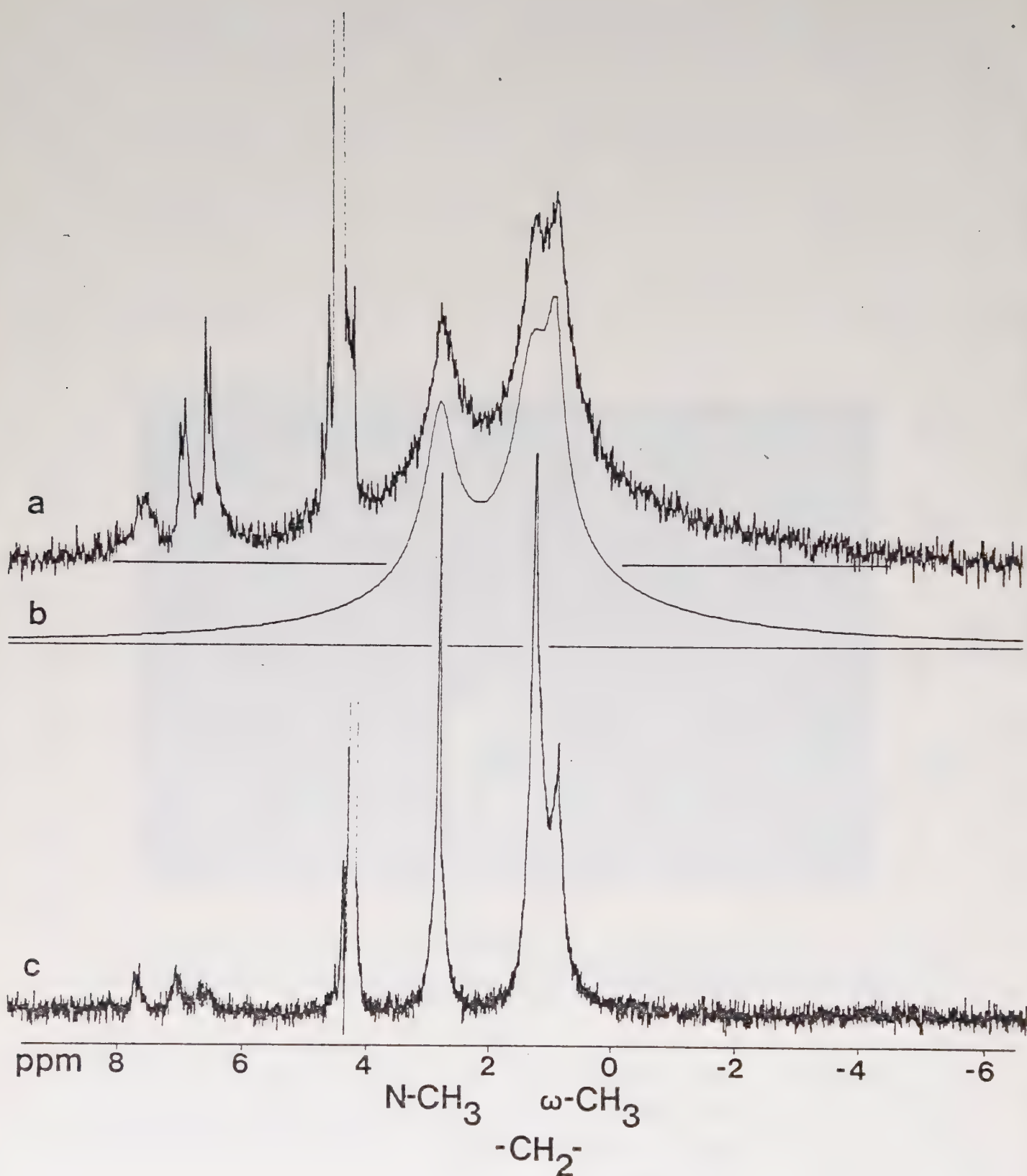


Fig. 3. 100 MHz proton NMR spectra of 12 mM solutions of a) CTA-salicylate and c) CTA p-hydroxybenzoate. The curve in b) is a simulation of spectrum a.



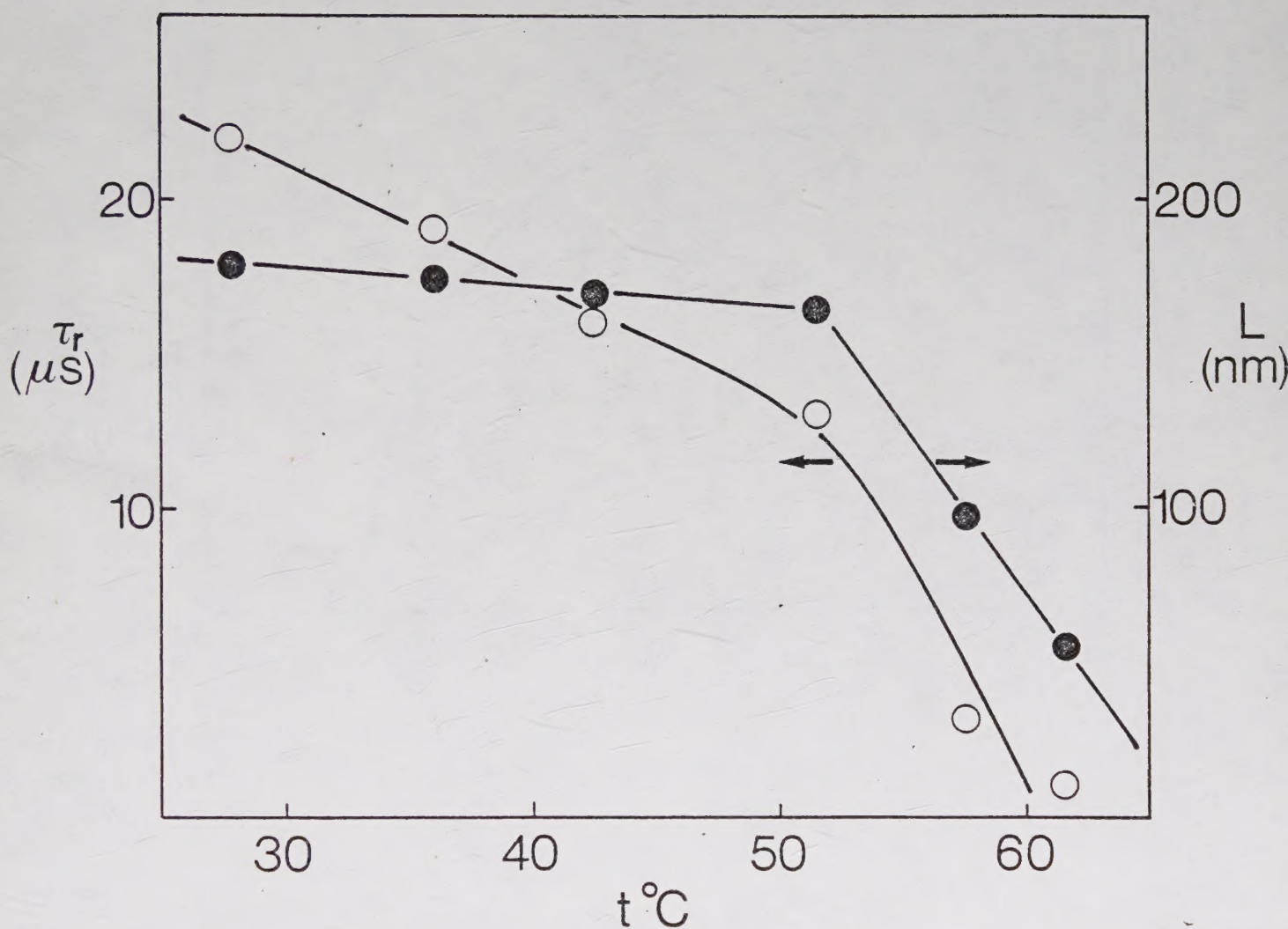


Fig. 4. The reorientational correlation time  $\tau_r$  (left hand scale) and the calculated length of the rods (right hand scale) for a 12 mM CTA-salicylate solution as a function of the temperature.



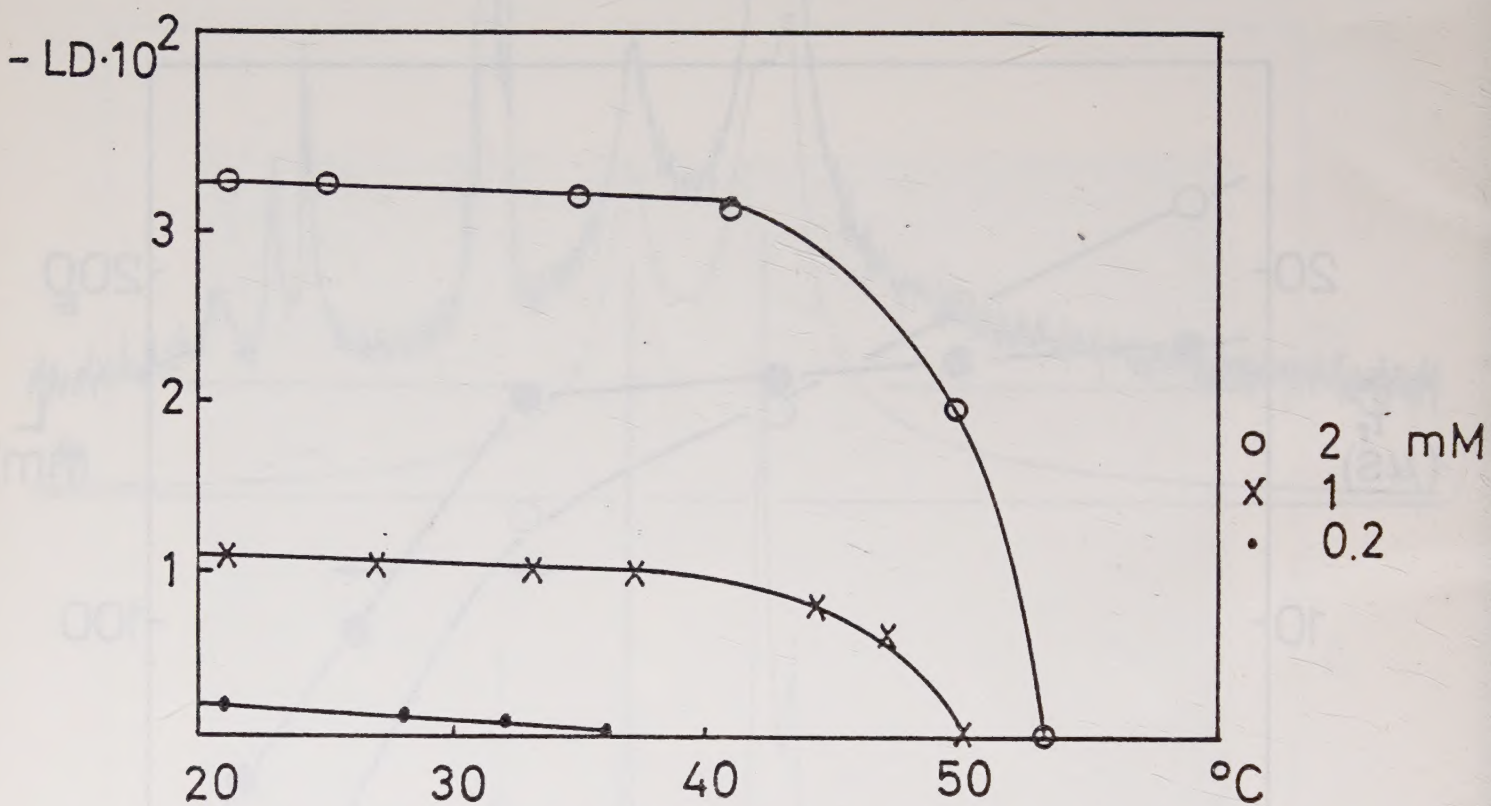


Fig. 5. The relative linear dichroism ( $LD/A$ ) as a function of temperature for three different CTA-salicylate solutions.







